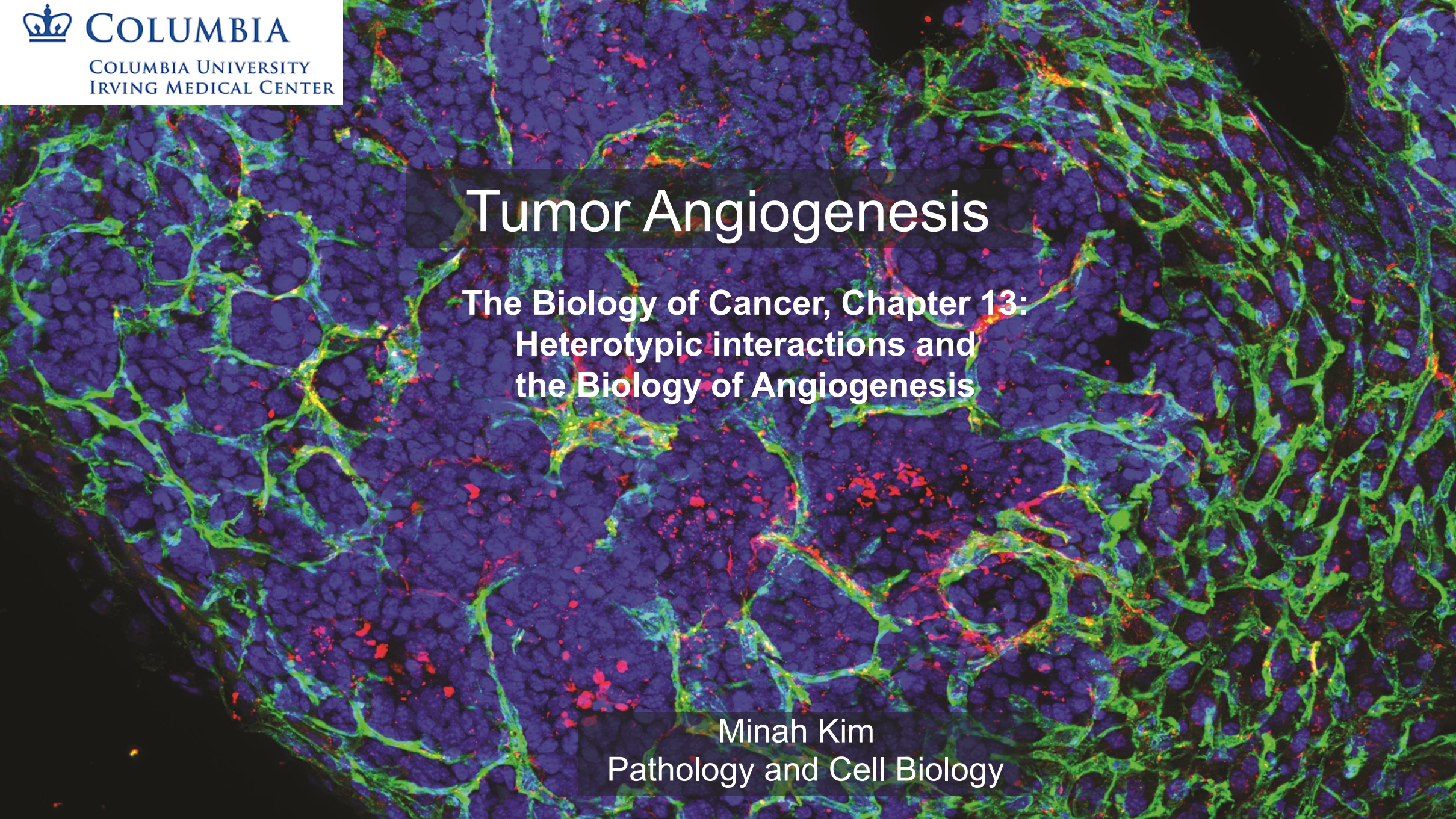


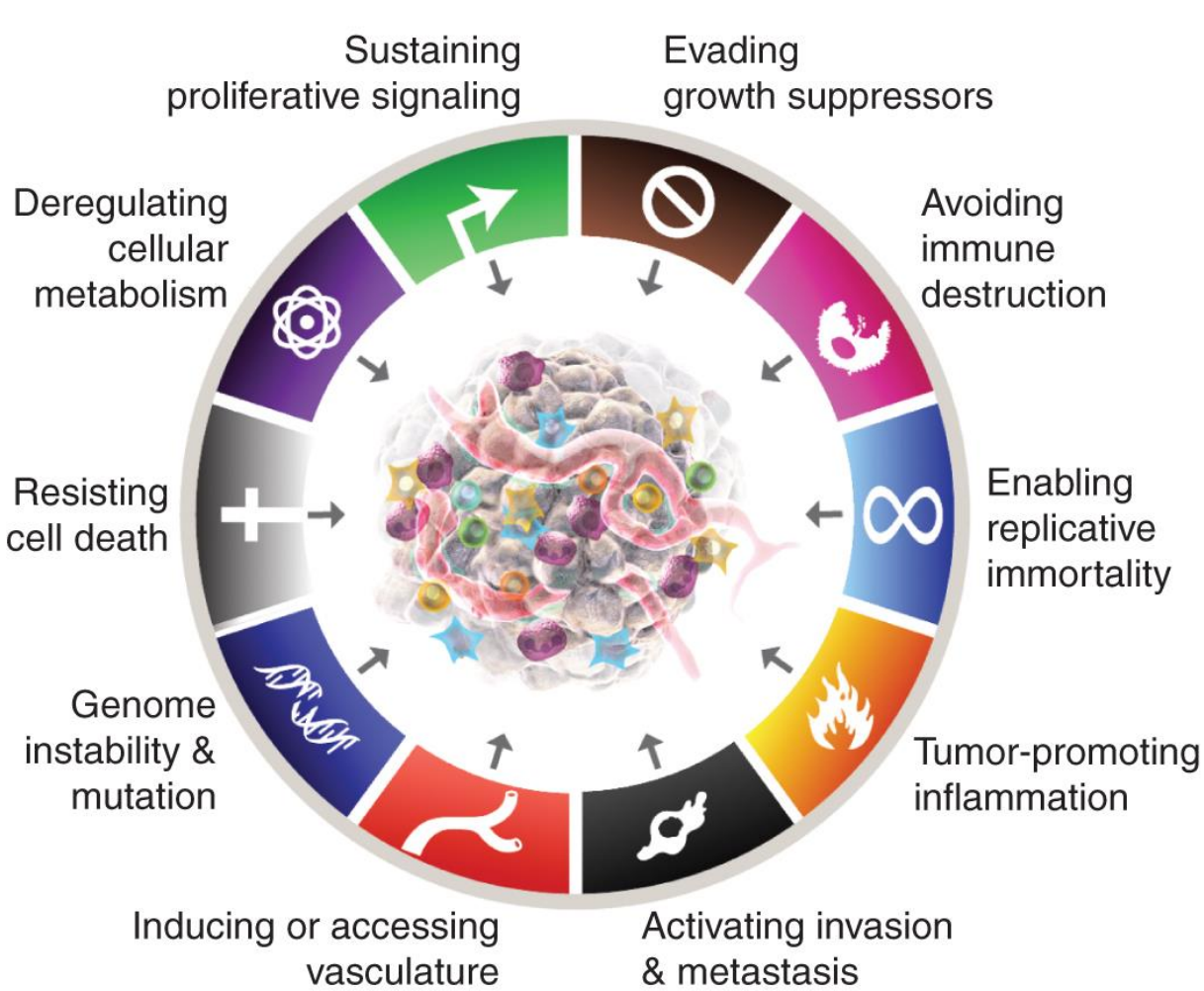
COLUMBIA
COLUMBIA UNIVERSITY
IRVING MEDICAL CENTER

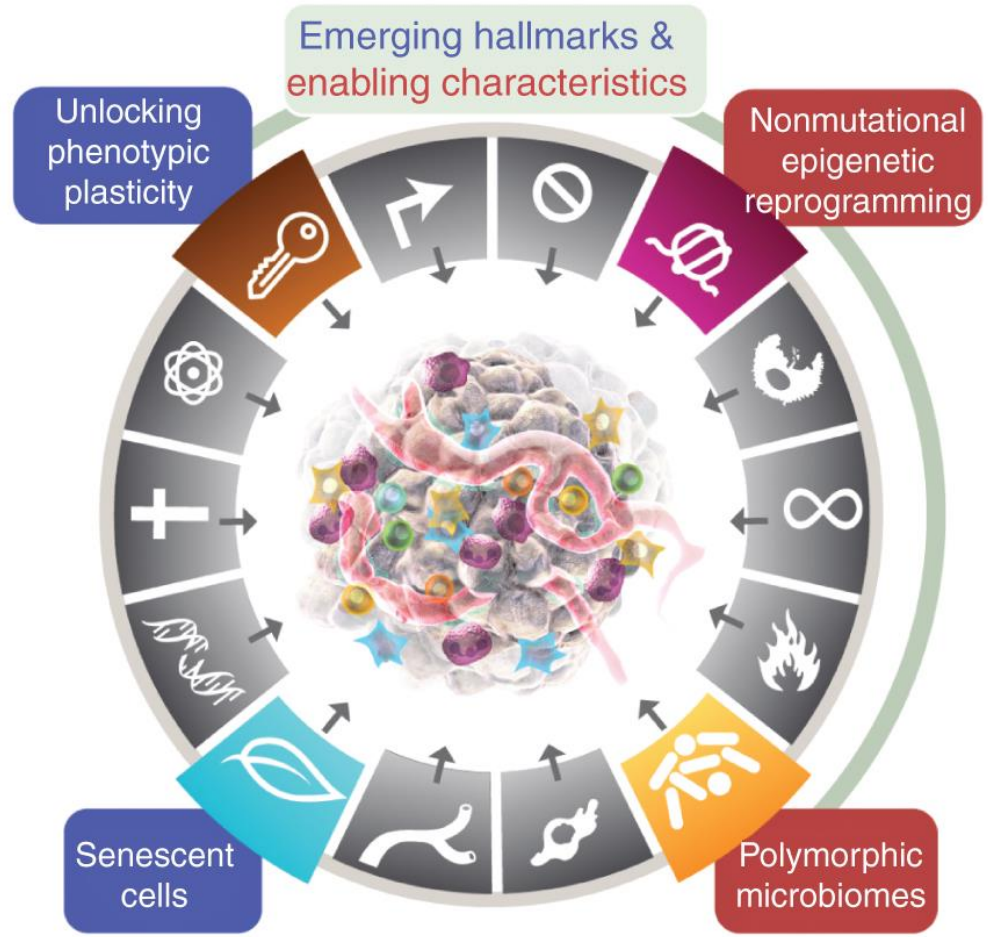
Tumor Angiogenesis

The Biology of Cancer, Chapter 13:
Heterotypic interactions and
the Biology of Angiogenesis

Minah Kim
Pathology and Cell Biology

The Hallmarks of Cancer

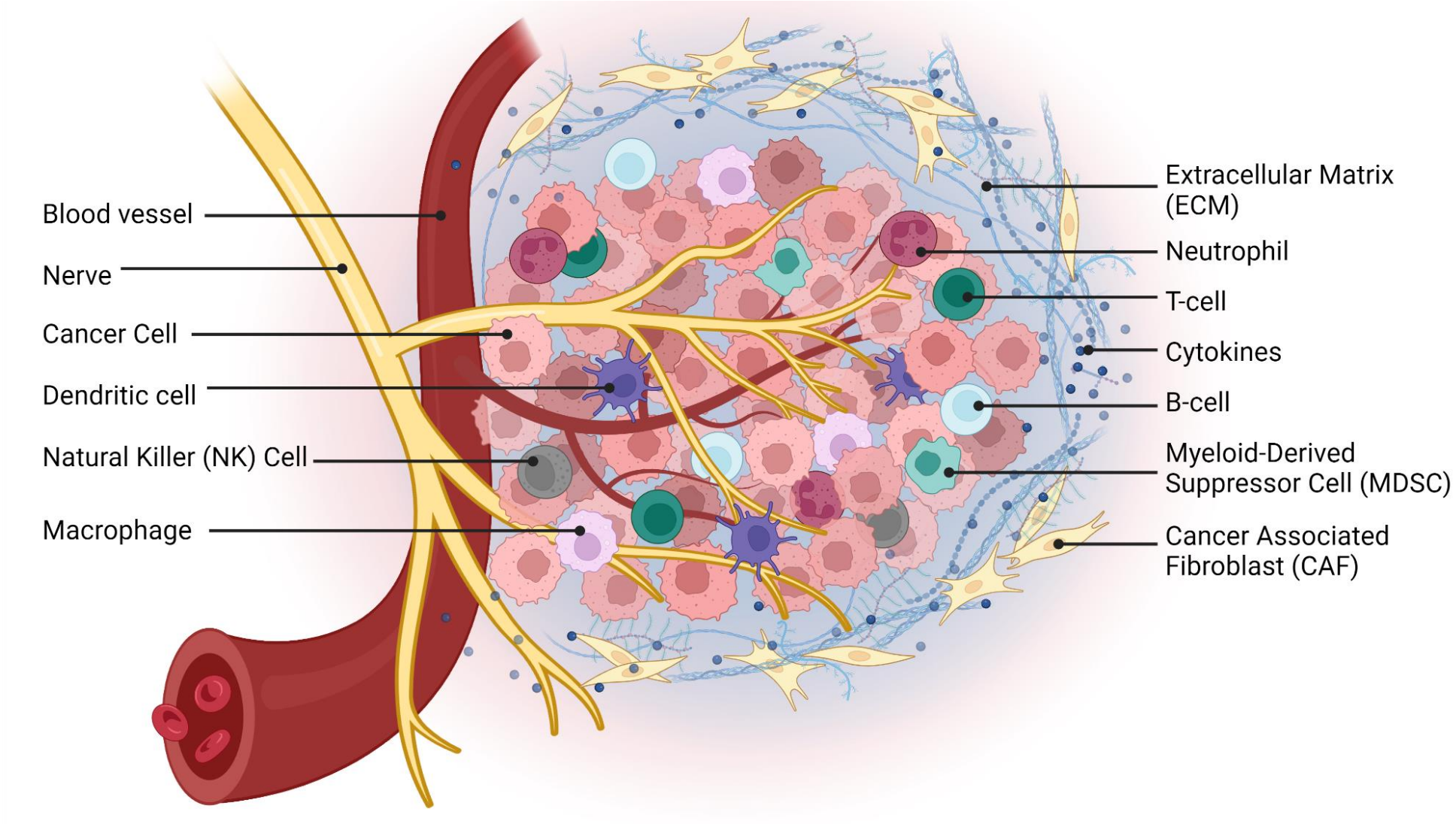




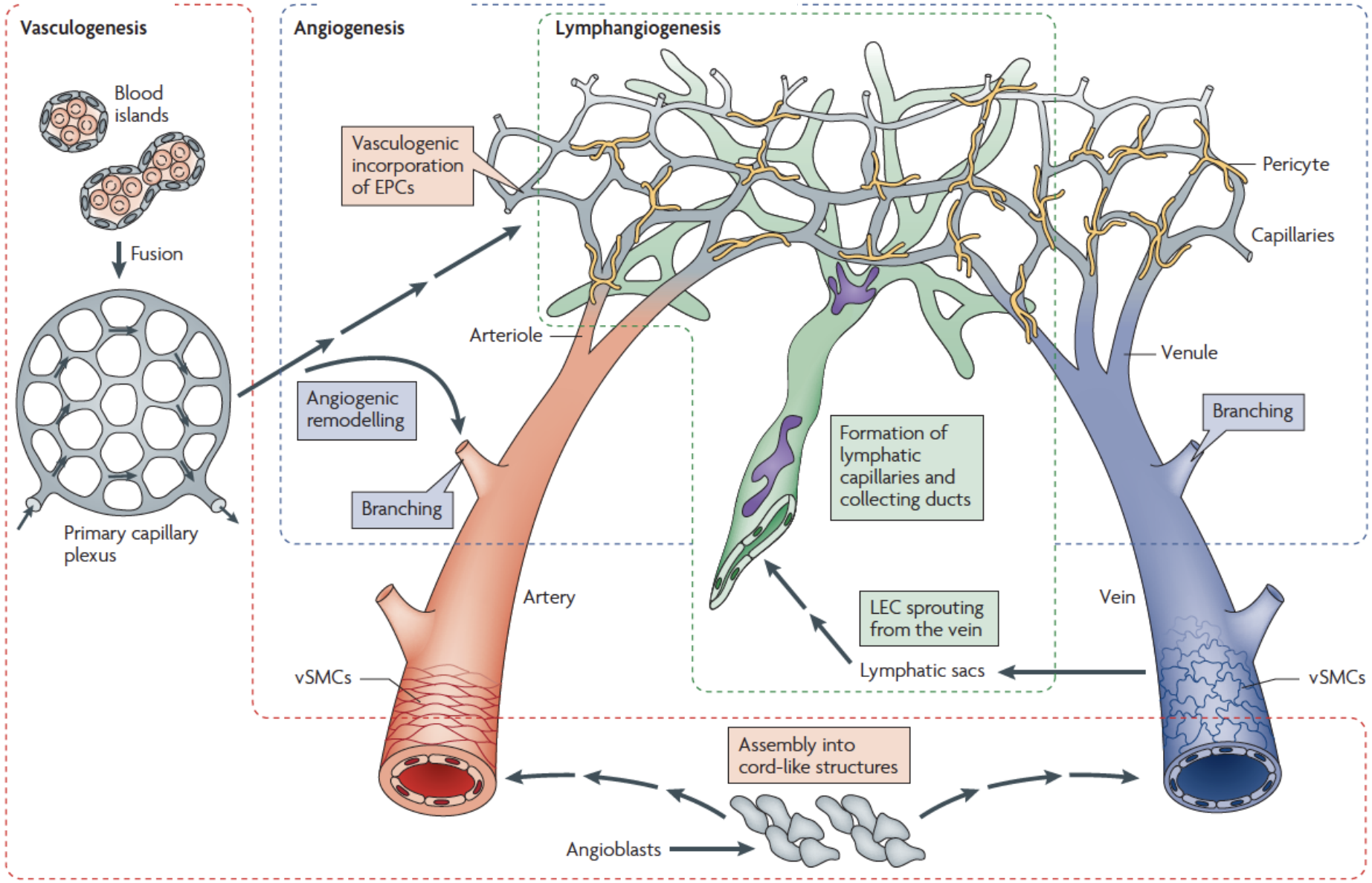
Cancer Discov. 2022;12(1):31-46. doi:10.1158/2159-8290.CD-21-1059

Hallmarks of Cancer: The Next Generation by *Douglas Hanahan, Robert A. Weinberg, Cell*

The Tumor Microenvironment

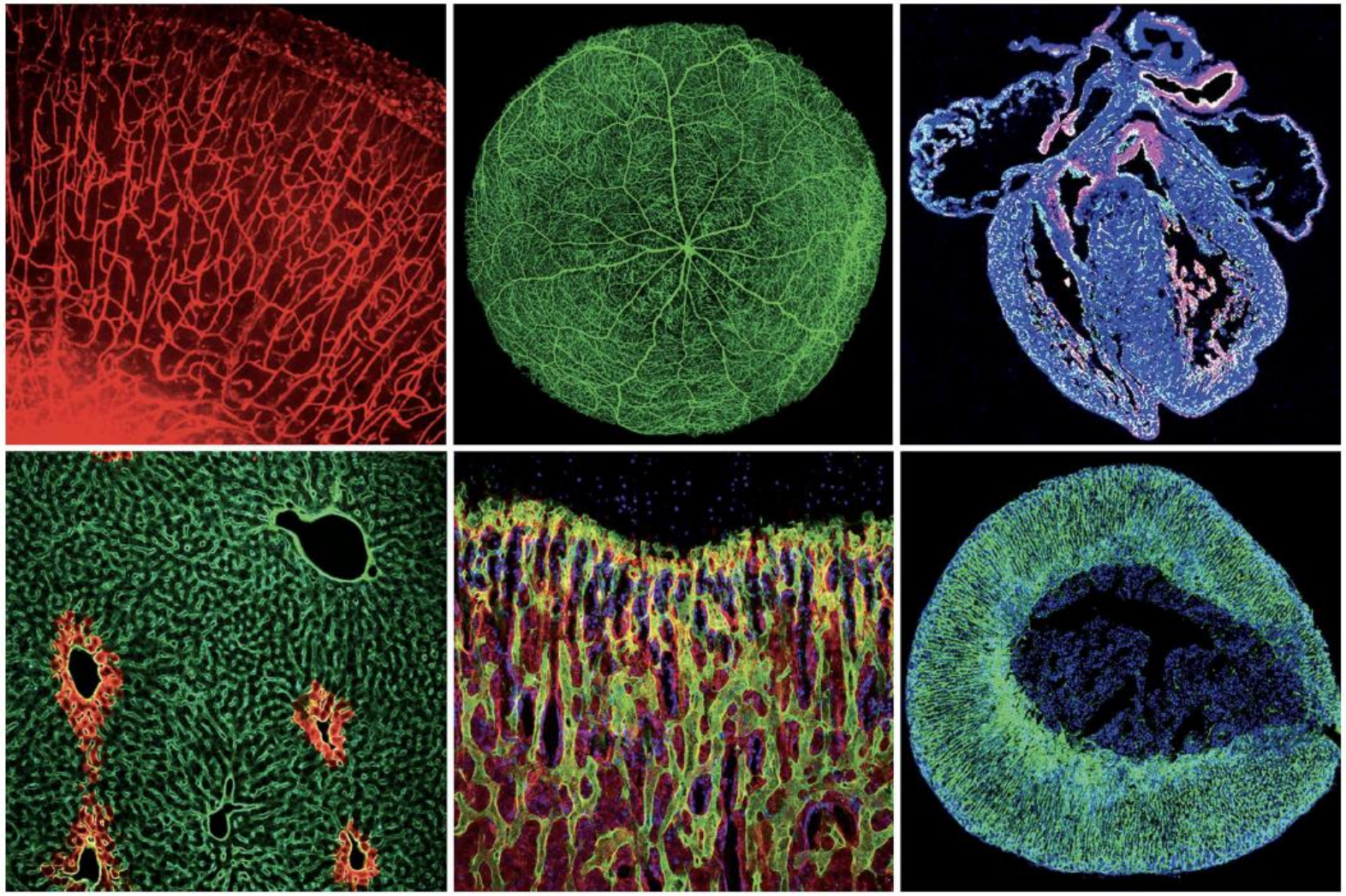


Vasculogenesis Angiogenesis Lymphangiogenesis



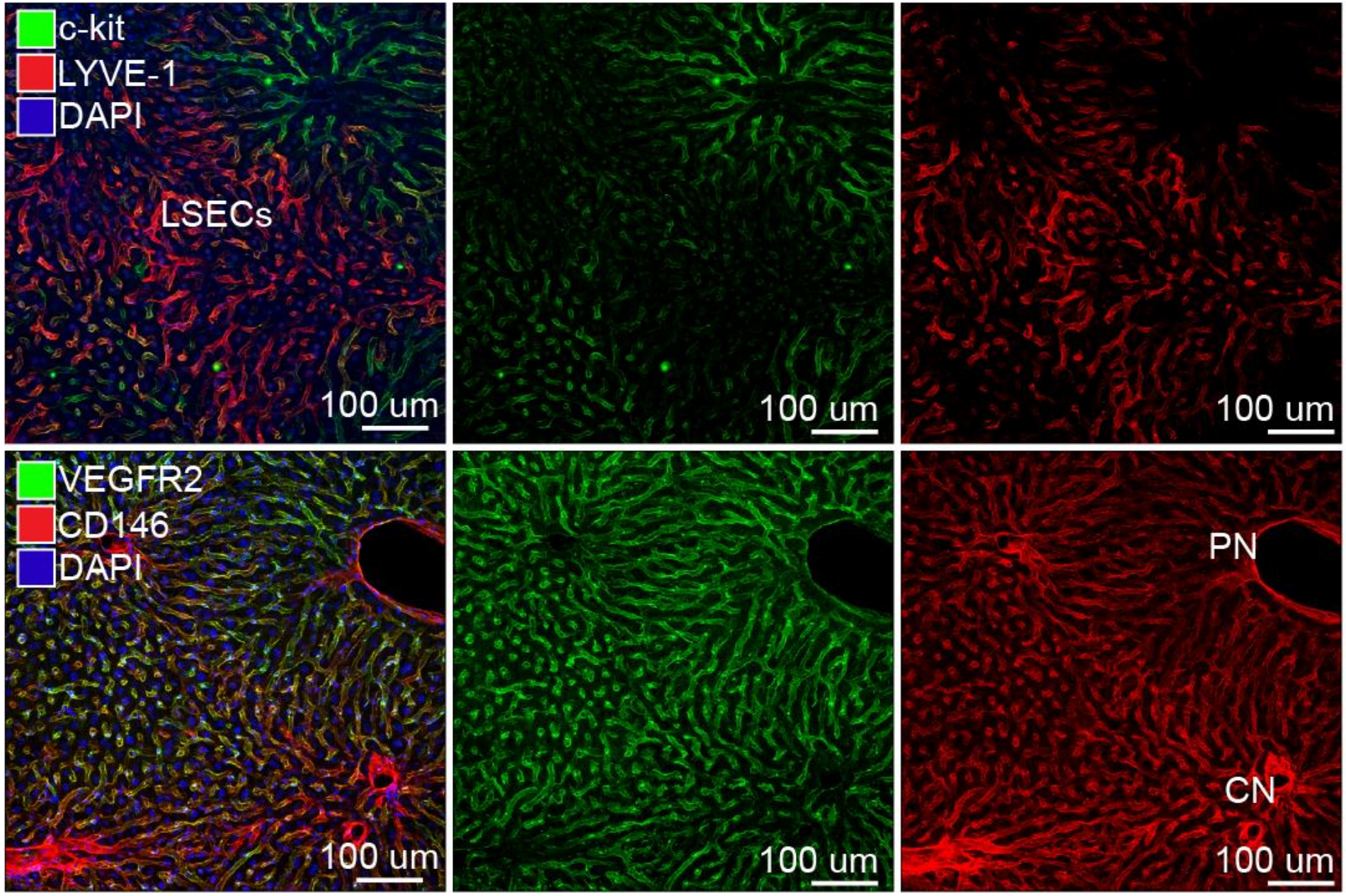
Ralf Adams and Kari Alitalo, Nature Reviews Molecular Cell Biology 2007

Organotypic vasculature: From descriptive heterogeneity to functional pathophysiology



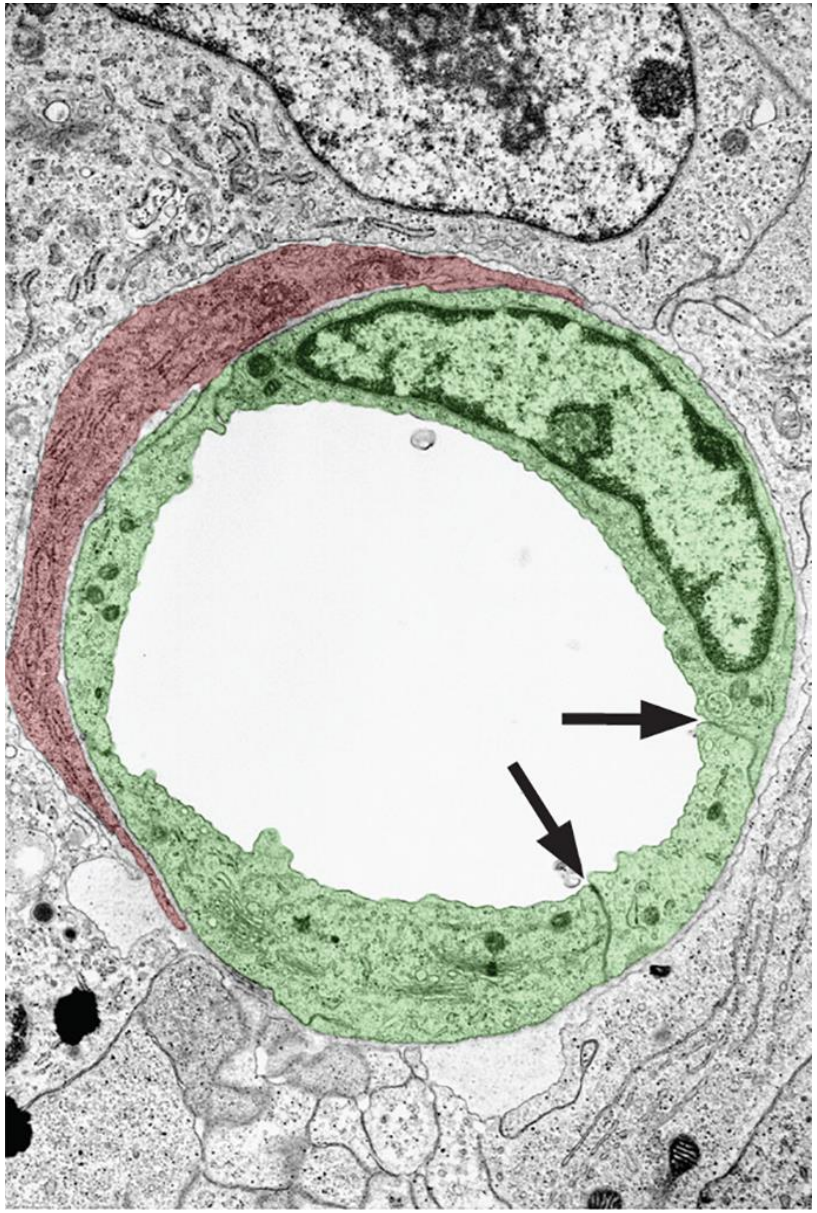
Hellmut Augustin and Gou Young Koh, Science 2017

Vascular phenotypes in healthy liver



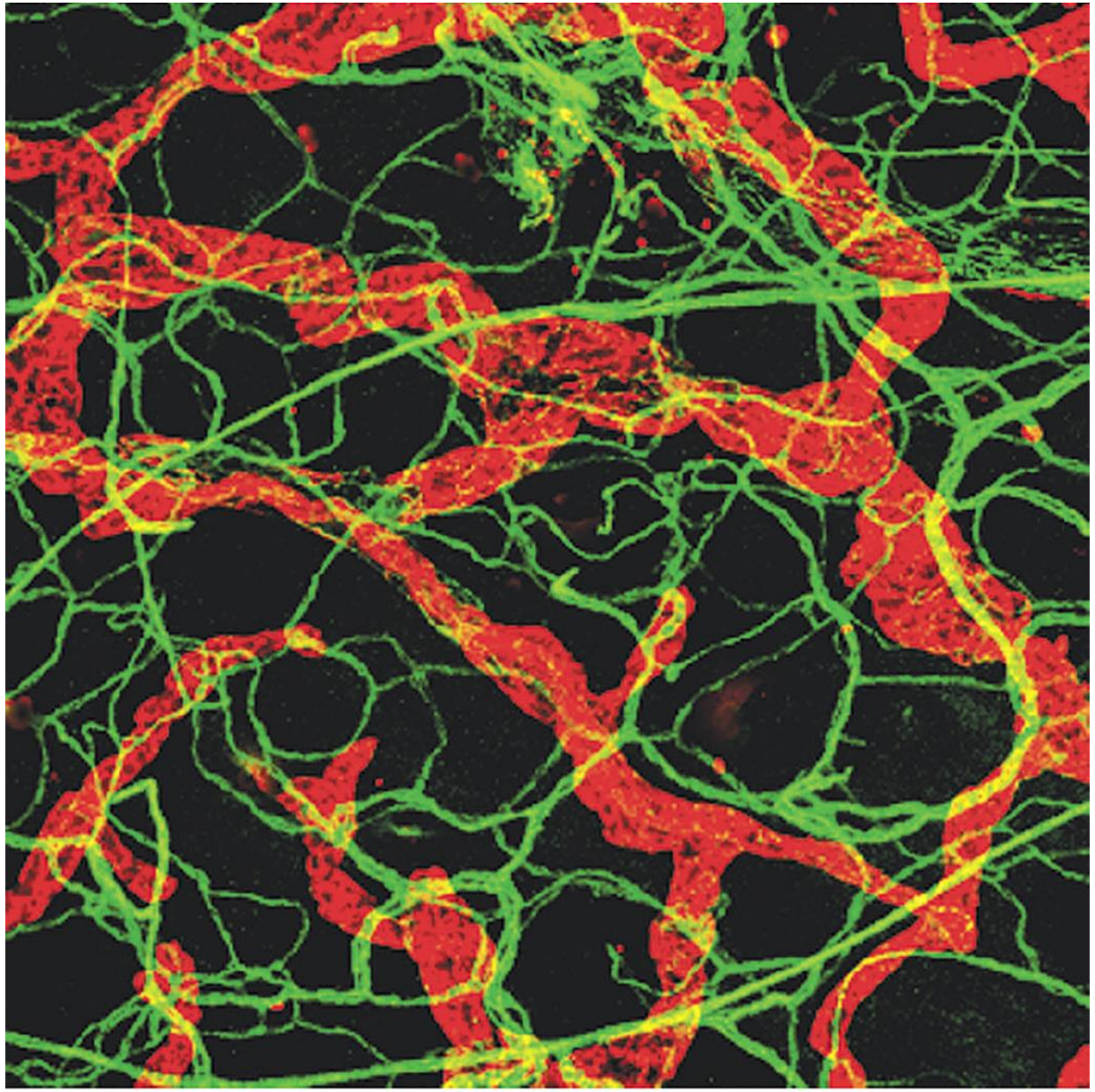
Comparison of BBB and LSEC Endothelium

Feature	BBB Endothelial Cells	Liver Sinusoidal Endothelial Cells (LSECs)
Barrier property	Extremely tight barrier	Highly permeable
Junctions	Continuous tight junctions (claudins, occludin, ZO-1) that prevent paracellular diffusion	Discontinuous or fenestrated with open pores (100–200 nm) allowing plasma exchange
Basement membrane	Thick and continuous	Discontinuous or absent
Transport mechanisms	Selective transporters (e.g., GLUT1, ABC transporters) tightly regulate entry of nutrients	Nonselective diffusion of solutes and macromolecules
Pericytes & astrocytes	Supported by pericytes and astrocyte end-feet, forming a neurovascular unit	No astrocytic coverage; surrounded by hepatocytes and Kupffer cells
Main function	Protects the brain by maintaining homeostasis and excluding toxins	Facilitates exchange for metabolism and detoxification



endothelial cells pericyte

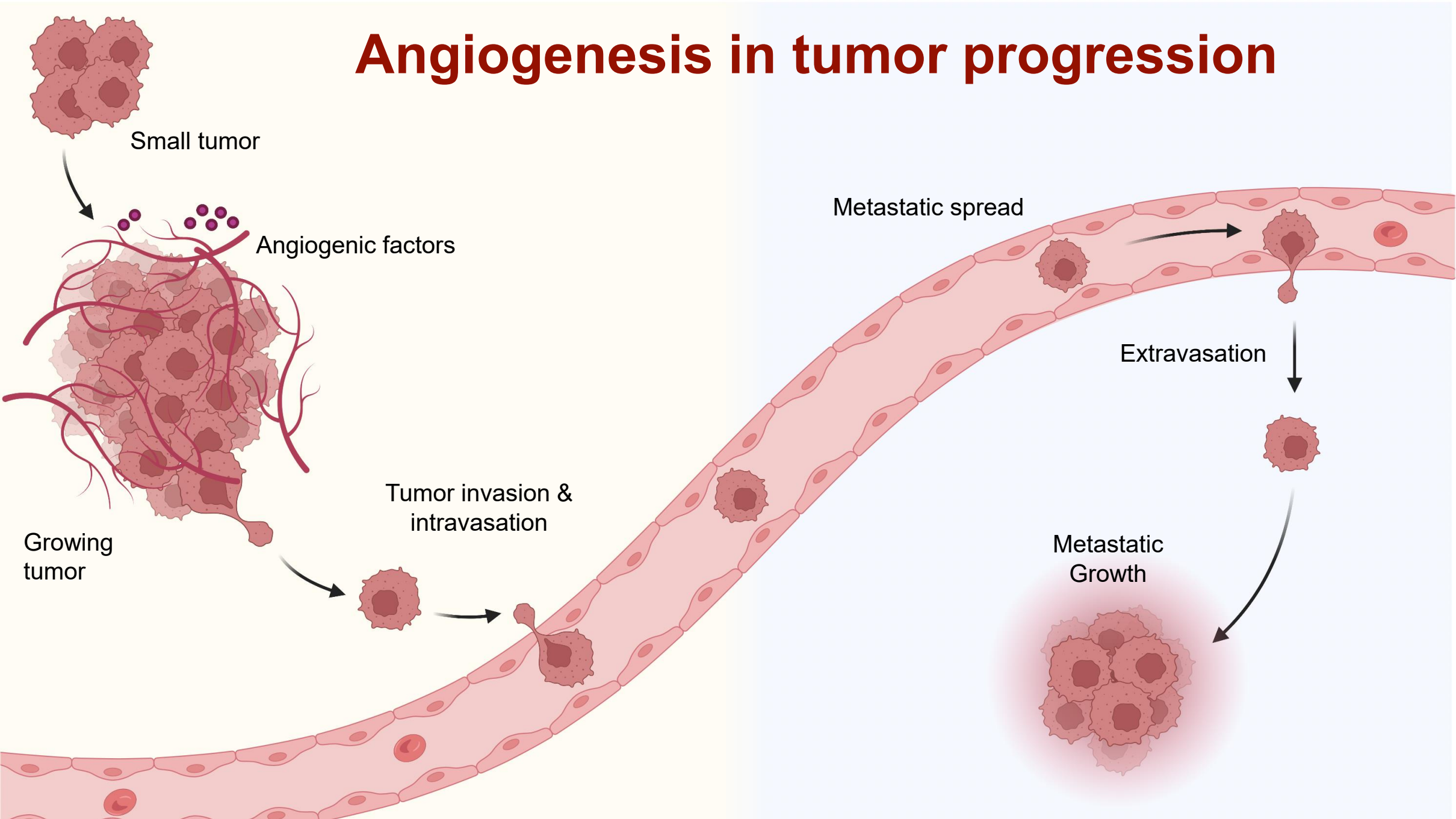
From B. Sennino et al., *Cancer Res.* 67:7358–7367, 2007.
With permission from American Association for Cancer Research.



capillaries lymph ducts

Courtesy of T. Tammela and K. Alitalo.

Angiogenesis in tumor progression



Angiogenesis

Definition: Growth of new blood vessels from existing ones

Relevance: Necessary for tumor growth

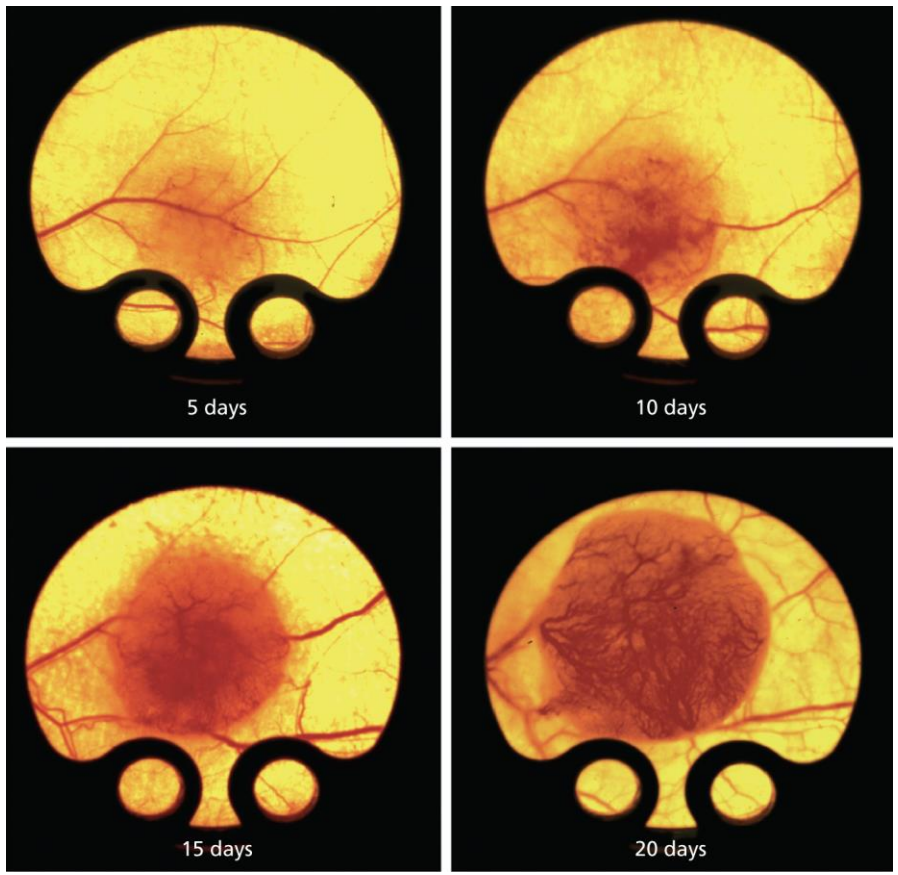
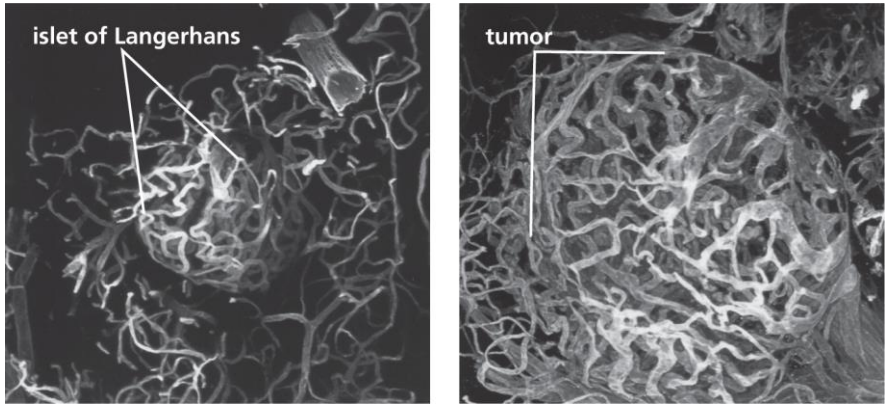


Figure 13.30 Recruitment of capillaries by an implanted tumors

Tumor vascularization

1. Angiogenesis : the formation of new blood vessels
2. Vascular co-option: use of preexisting vessels
3. Vascular mimicry: transdifferentiation of cancer cells to endothelial cells

The angiogenic switch is essential for tumor expansion



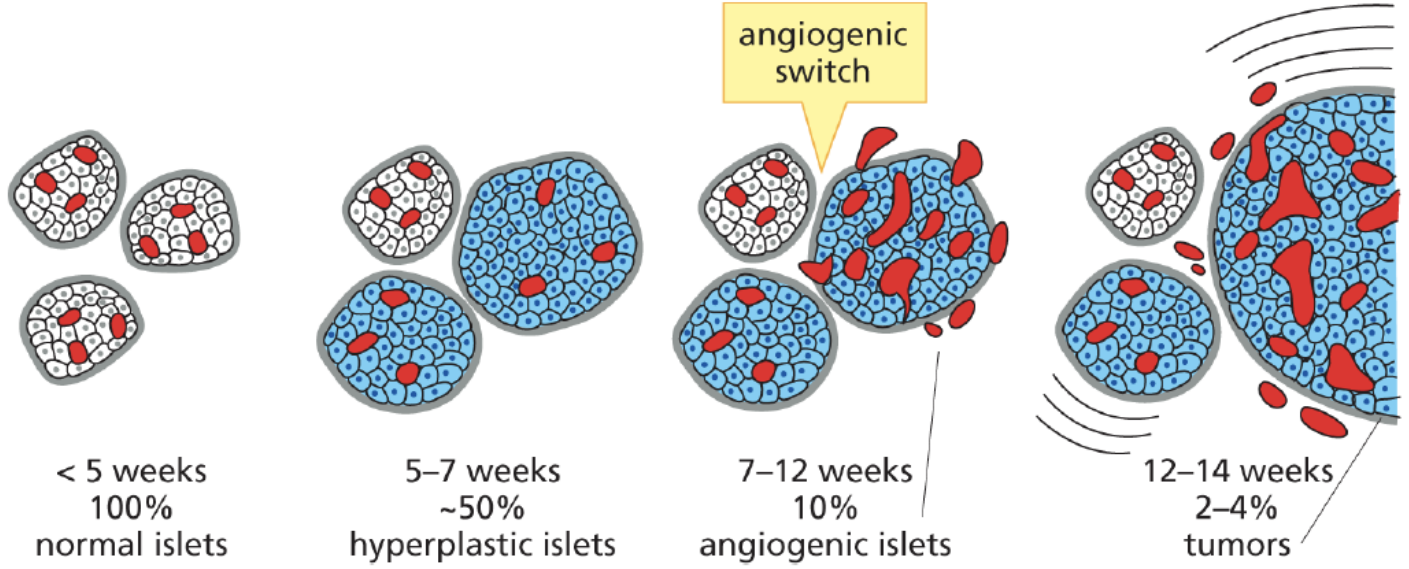
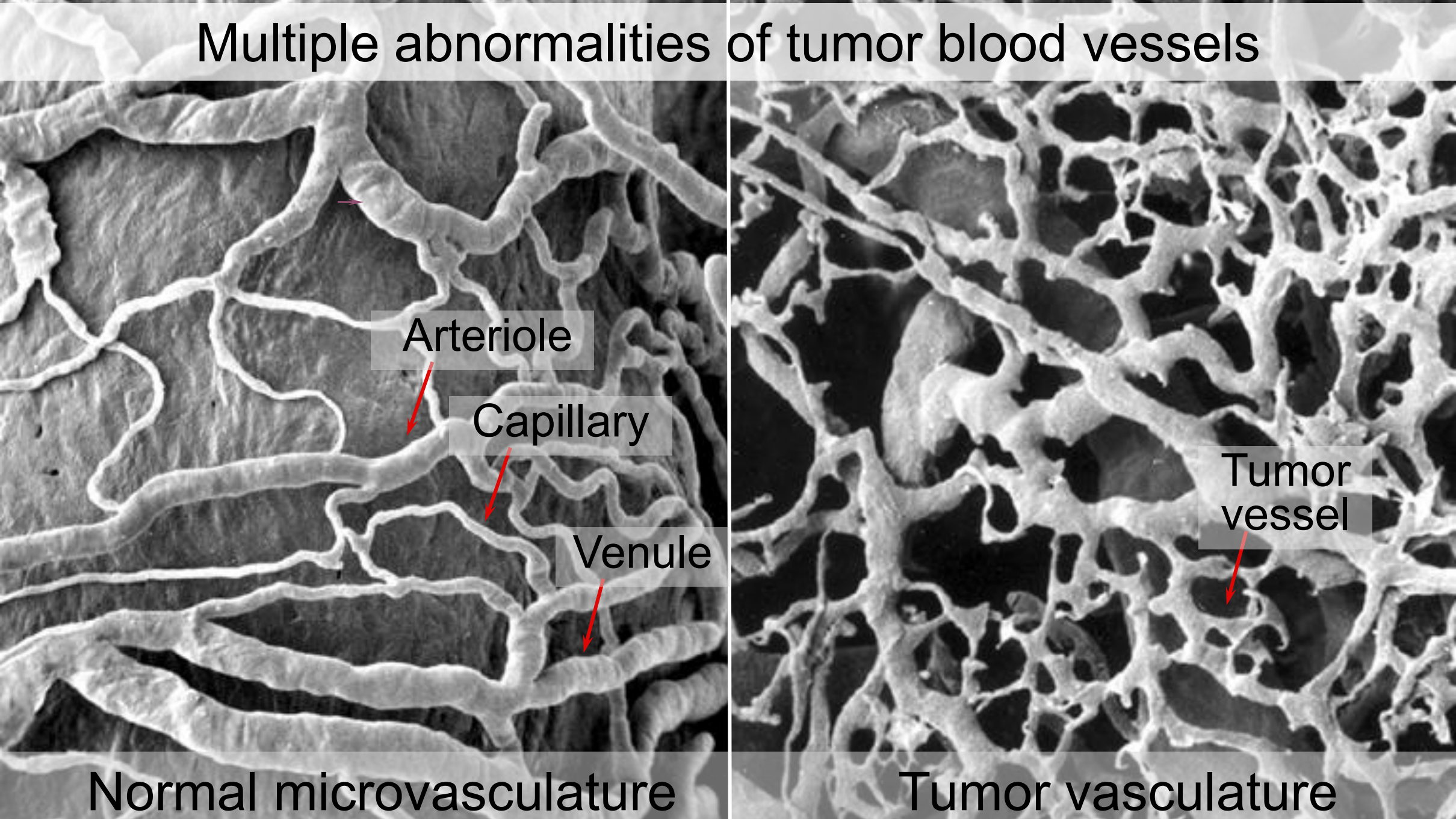
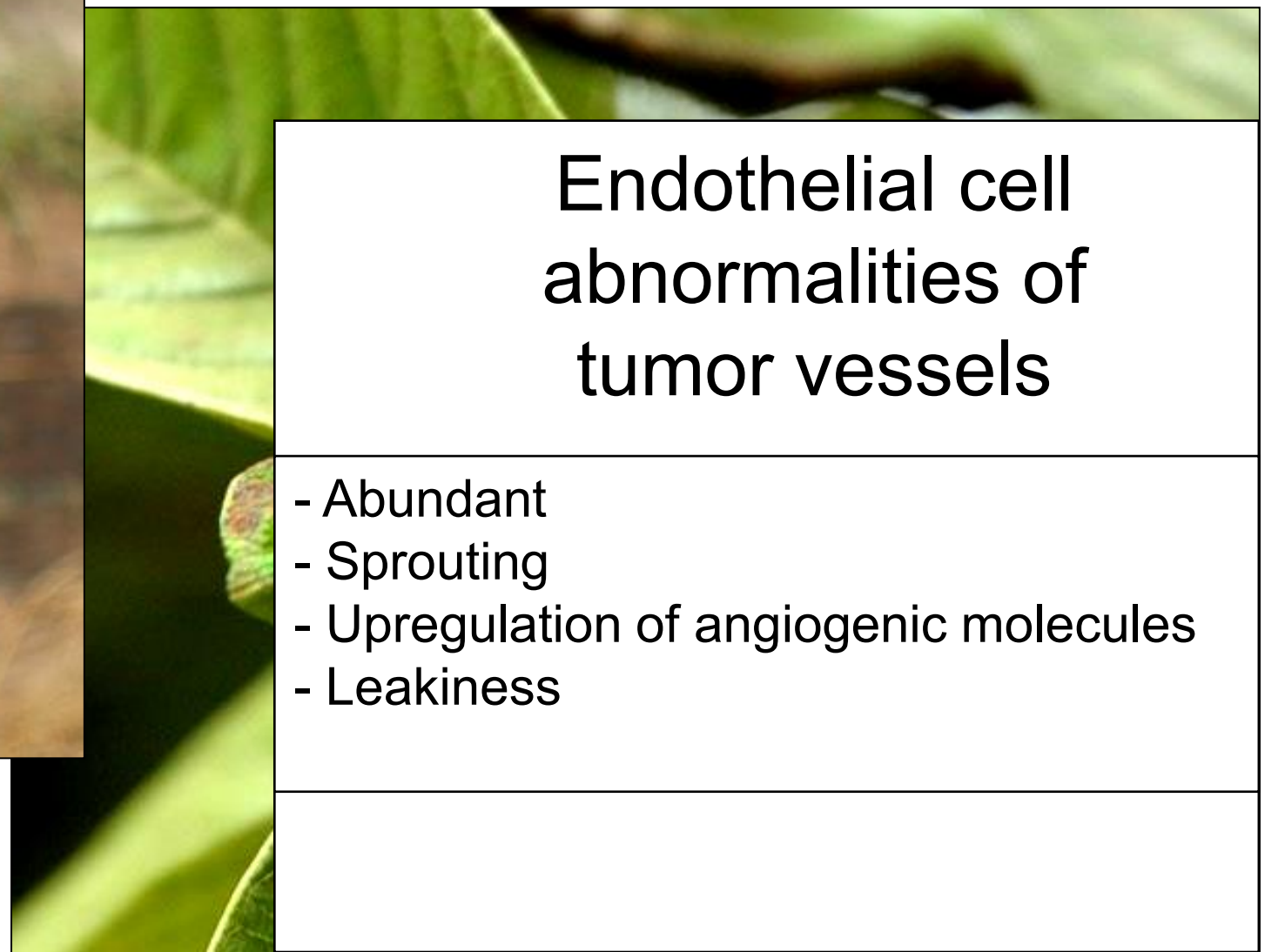


Figure 13.35 The Rip-Tag model of islet cell tumor progression.

Multiple abnormalities of tumor blood vessels



Tumor vessels are abnormal because they adapt to the abnormal microenvironment in tumors

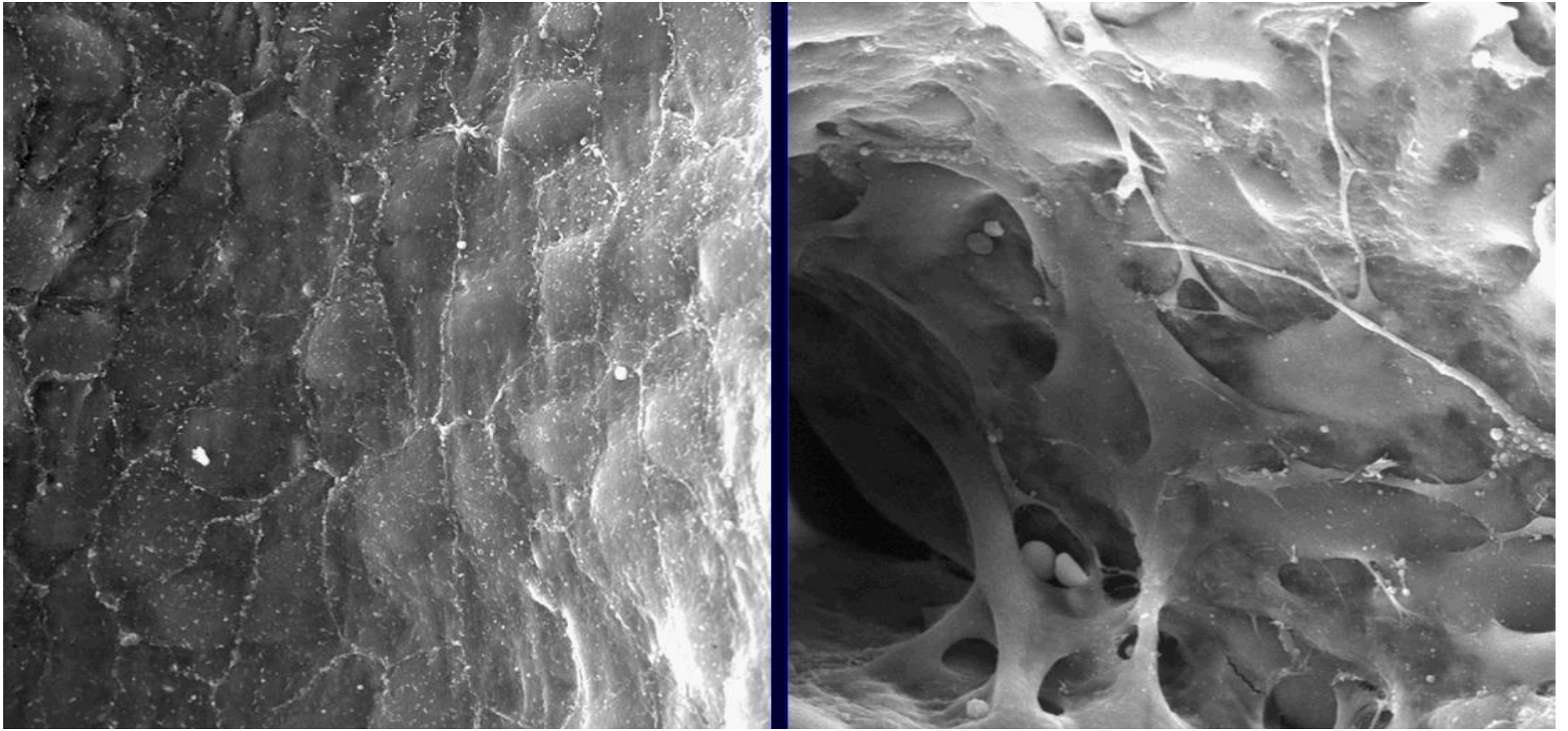


Endothelial cell abnormalities of tumor vessels

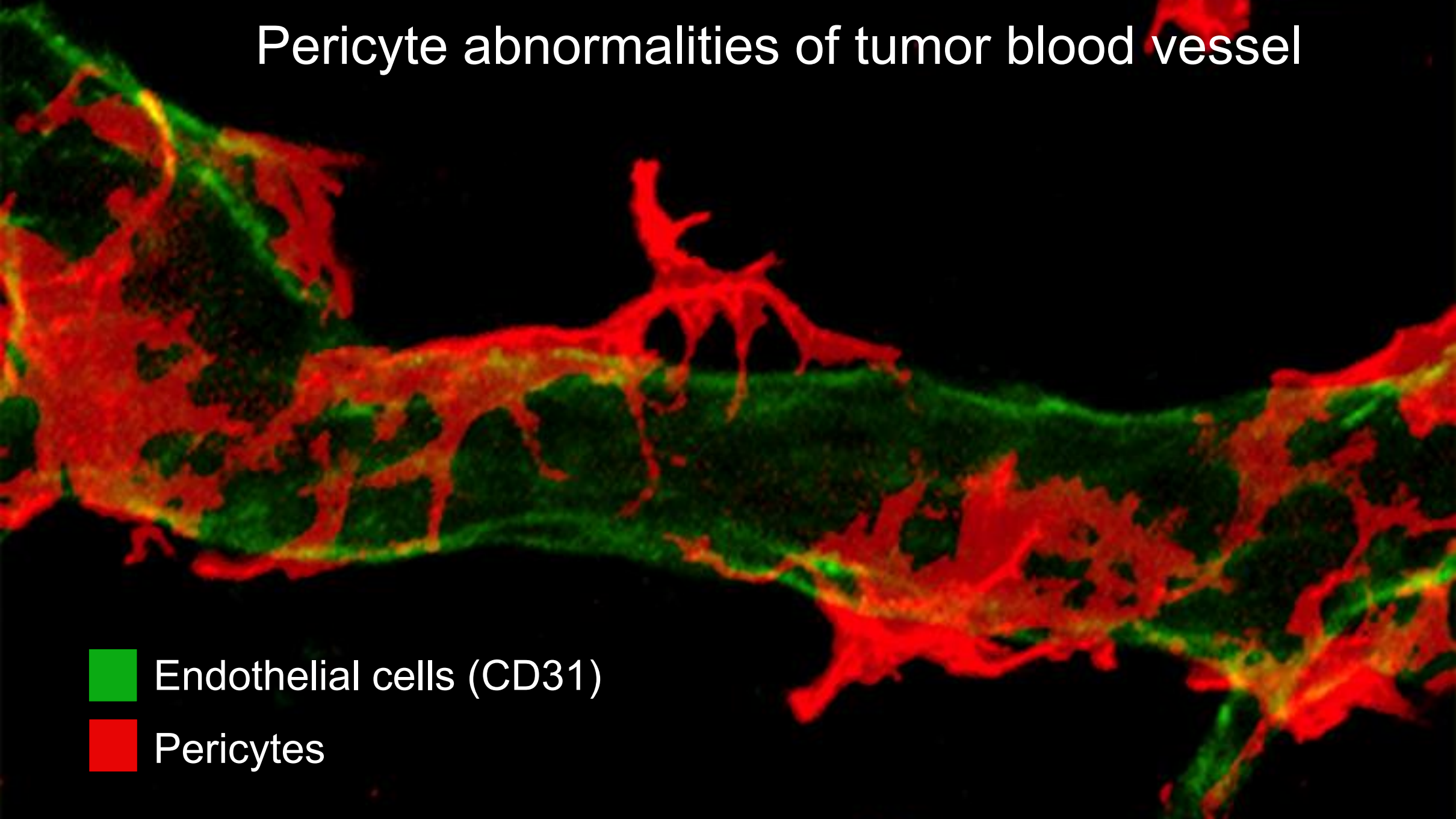
- Abundant
- Sprouting
- Upregulation of angiogenic molecules
- Leakiness

Normal blood vessel

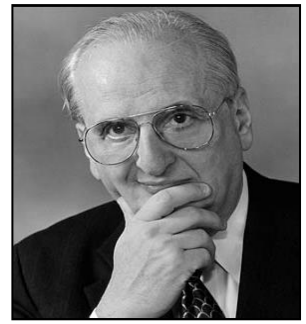
Tumor blood vessel



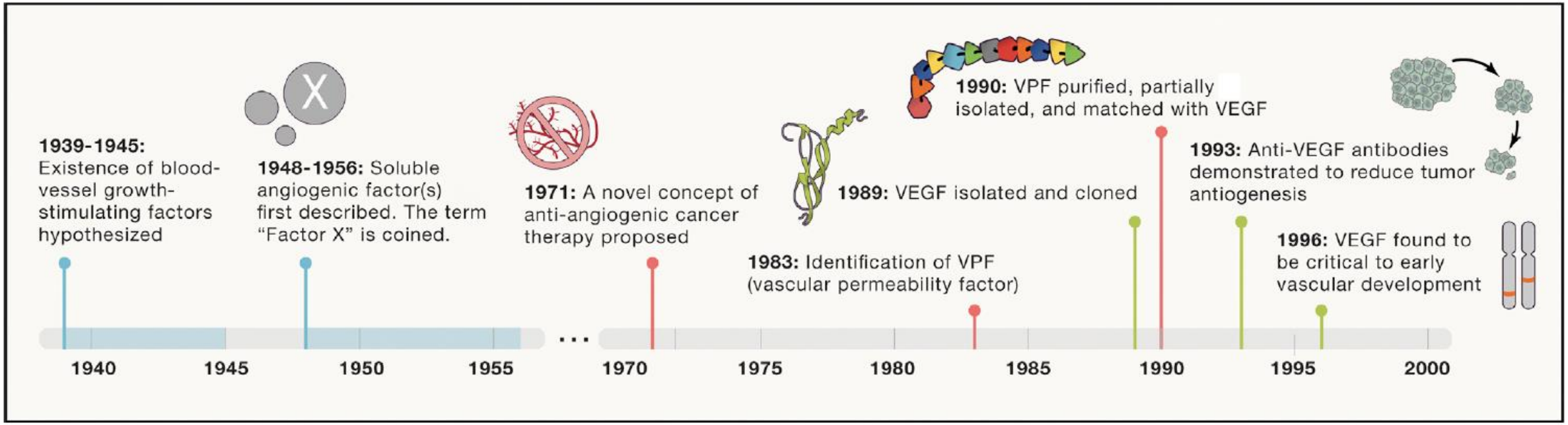
Luminal (inside) surface of blood vessel endothelium


Pericyte abnormalities of tumor blood vessel
Endothelial cells (CD31)
Pericytes

A Timeline of VEGF Discovery



Judah Folkman (1933-2008)

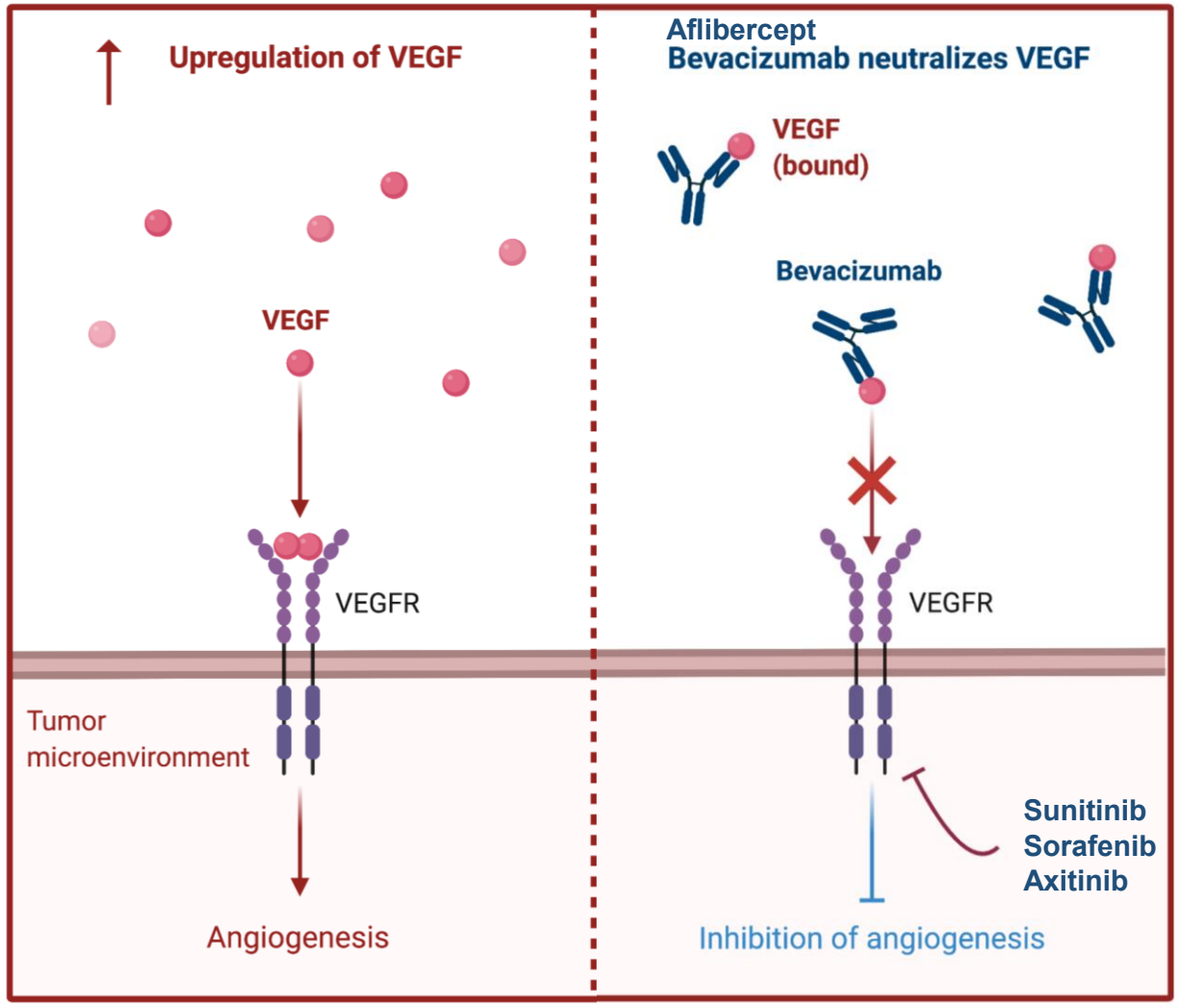


Apte et al., Cell Review 2019

Effects of angiogenesis inhibitors

- On blood vessels? Stop the growth of new blood vessels
- On tumor cells? Stop tumor growth

Targeting VEGF family members and receptors



VEGF as a vascular permeability modulator

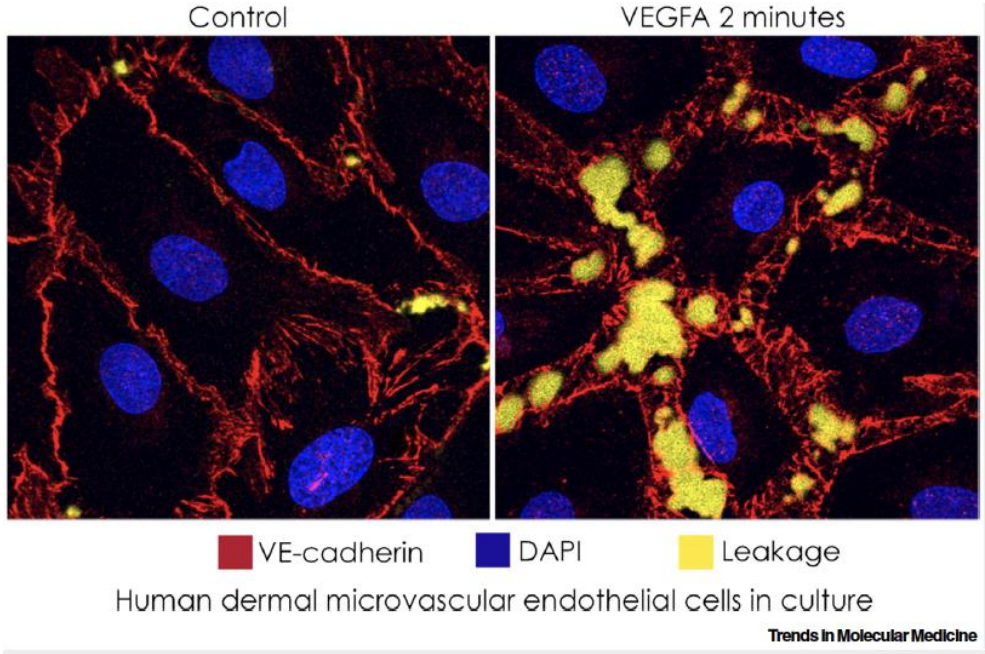


Figure I. Comparison of Control and VEGFA-Stimulated Microvascular Endothelial Cells. Leakage is detected by fluorescent streptavidin (yellow) bound to biotin in the substrate in an *in vitro* vascular permeability imaging assay (Merck KGaA, Darmstadt). Separations of junctions in cultured human dermal microvascular endothelial cells exposed to VEGFA are larger and more numerous than occur *in vivo*.

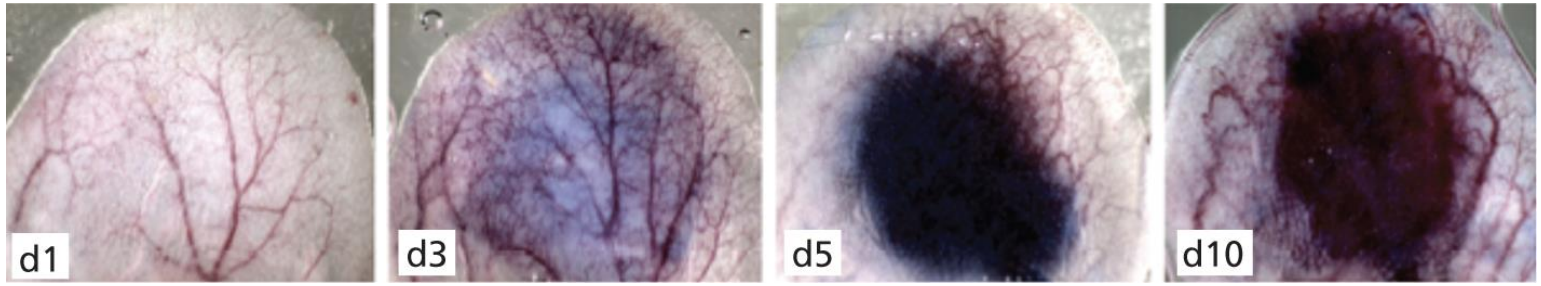


Figure 13.33 Capillary permeability following an adenoviral expression of VEGF-A

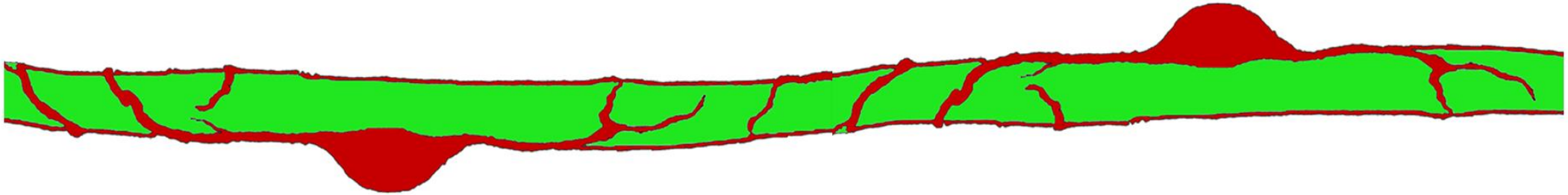
FDA-approved drugs targeting VEGF-regulated pathways in oncology (in combination with other therapies)

Adapted from Zirlik and Duyster (2018).

1. Bevacizumab (target: VEGF-A): locally, advanced, metastatic, or recurrent colorectal cancer (CRC); metastatic NSCLC; recurrent glioblastoma; cervical cancer; certain recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer; metastatic RCC
2. Ziv-aflibercept (targets: VEGF-A, VEGF-B, PlGF): metastatic CRC
3. Ramucirumab (target: VEGFR2): metastatic CRC, metastatic NSCLC, gastric or gastroesophageal adenocarcinoma
4. Multiple TKIs (sorafenib, sunitinib, regorafenib, pazopanib, axitinib, vandetanib, lenvatinib, cabozantinib): various cancers depending on the specific TKI, including RCC, hepatic cell carcinoma, thyroid cancer, pancreatic neuroendocrine tumors, gastrointestinal stromal tumors, soft tissue sarcoma, medullary thyroid cancer

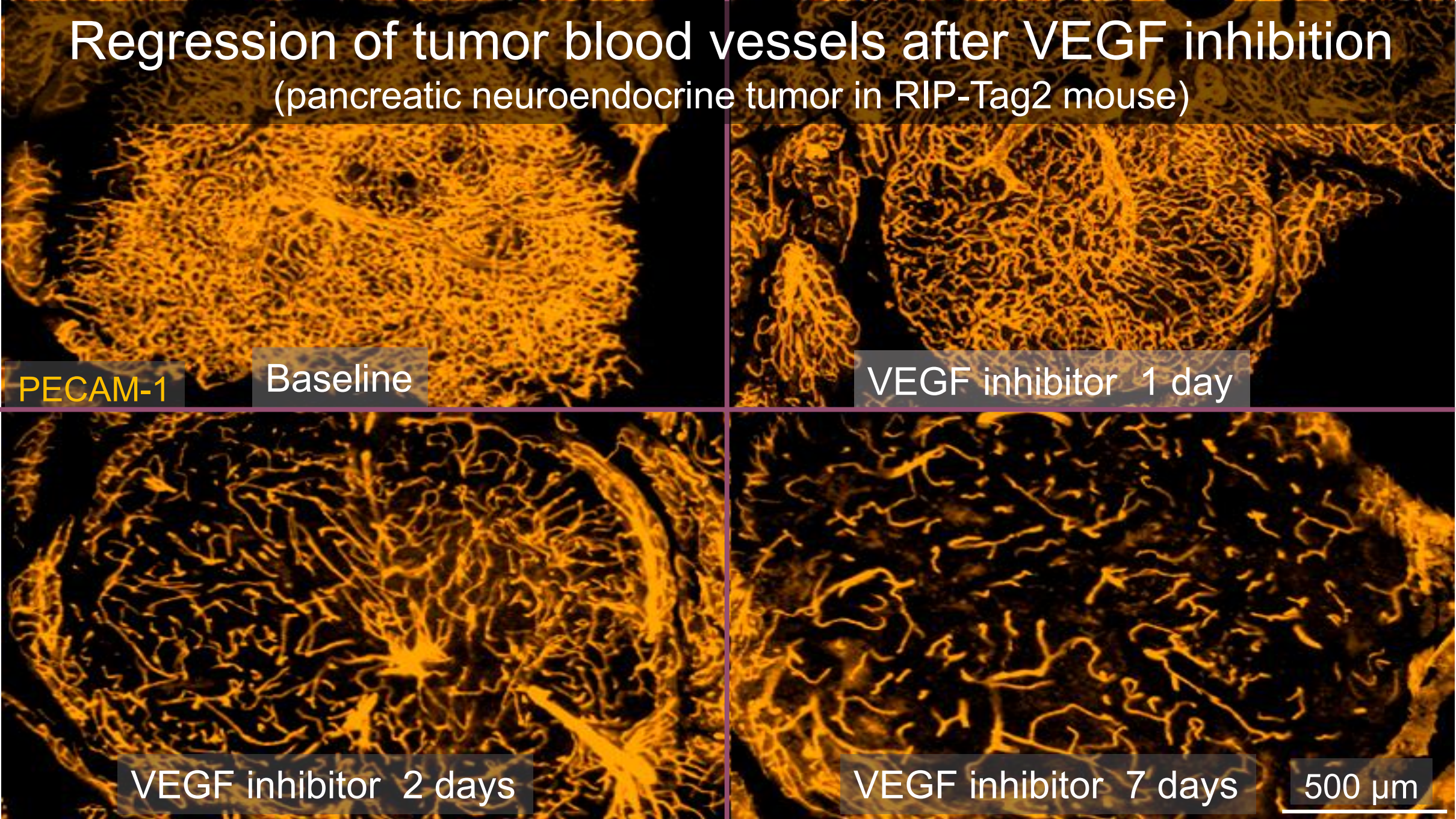
Apte et al., Cell Review 2019

Tumor vessels have abnormal endothelial cells and pericytes

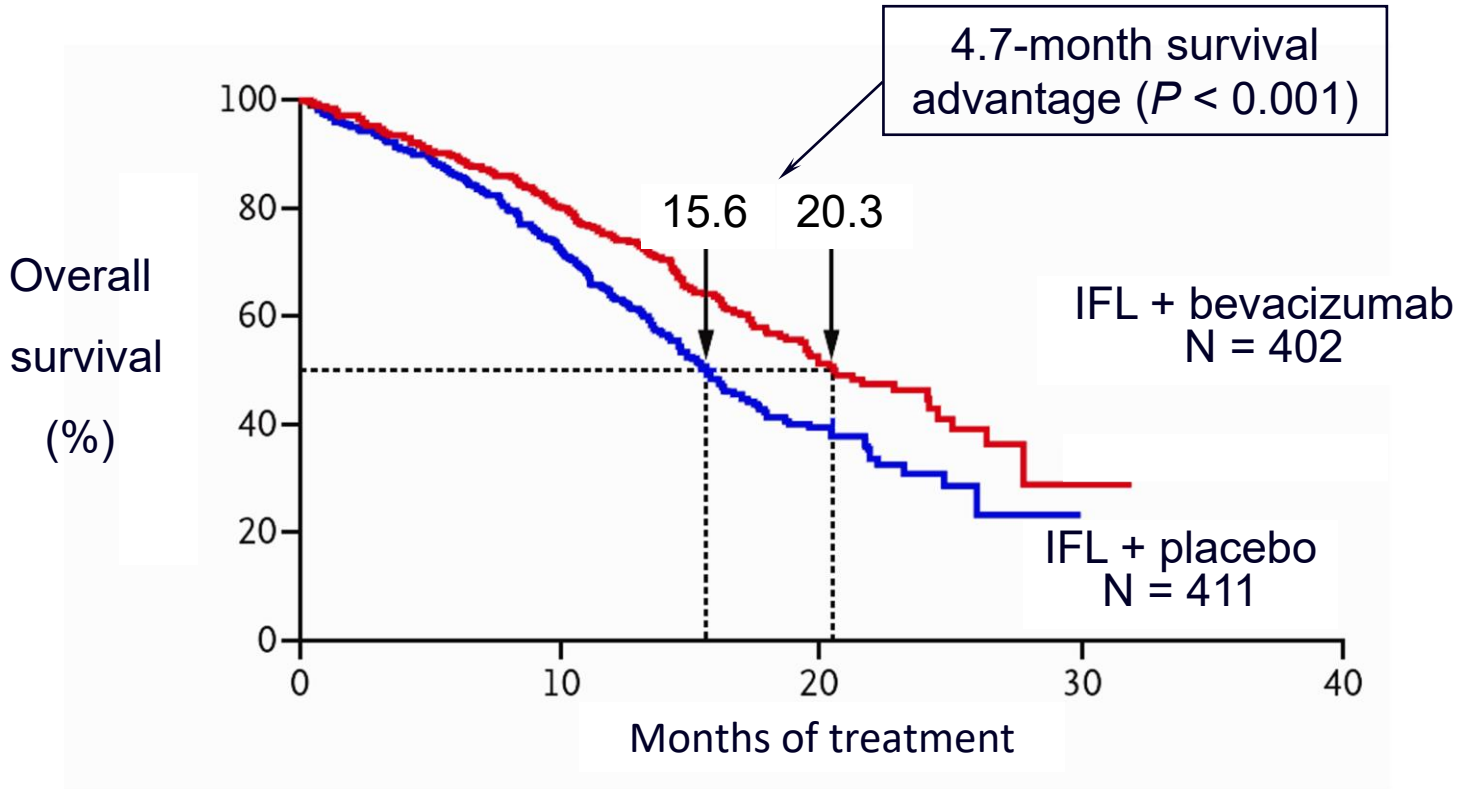


Normal capillary: Stable- insensitive to VEGF inhibitors

Tumor vessel: Unstable- sensitive to VEGF inhibitors


Regression of tumor blood vessels after VEGF inhibition
(pancreatic neuroendocrine tumor in RIP-Tag2 mouse)
PECAM-1
Baseline
VEGF inhibitor 1 day
VEGF inhibitor 2 days
VEGF inhibitor 7 days
500 µm

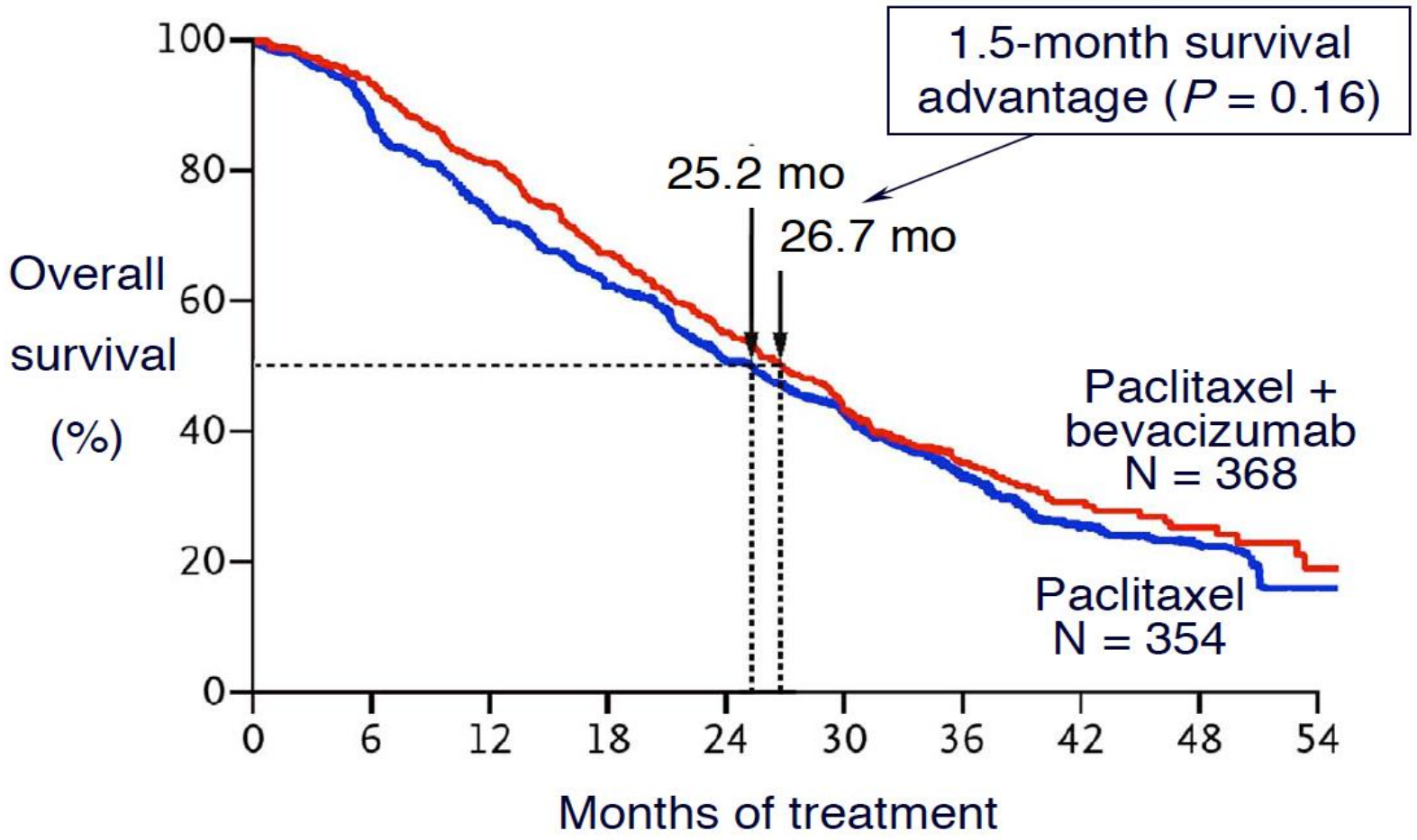
Survival advantage in metastatic colorectal cancer with anti-VEGF antibody treatment



IFL: Irinotecan, Fluorouracil, and Leucovorin

Hurwitz *et al*. *NEJM* 350: 2335-42, 2004

Anti-VEGF antibody action in metastatic breast cancer: No significant overall survival advantage



Miller et al. NEJM 357:2666-76, 2007

J Neurooncol (2010) 99:237–242
DOI 10.1007/s11060-010-0121-0

CLINICAL STUDY - PATIENT STUDY

Rebound tumour progression after the cessation of bevacizumab therapy in patients with recurrent high-grade glioma

Richard M. Zuniga · Roy Torcuator · Rajan Jain · John Anderson · Thomas Doyle · Lonni Schultz · Tom Mikkelsen

R. Torcuator · T. Mikkelsen
Department of Neurosurgery, Henry Ford Health System, Detroit, MI, USA

Annals of Oncology — New Editor-in-Chief Jan B. Vermorken

Annals of Oncology Advance Access originally published online on August 5, 2008
Annals of Oncology 2008 19(9):1659-1661; doi:10.1093/annonc/mdn540

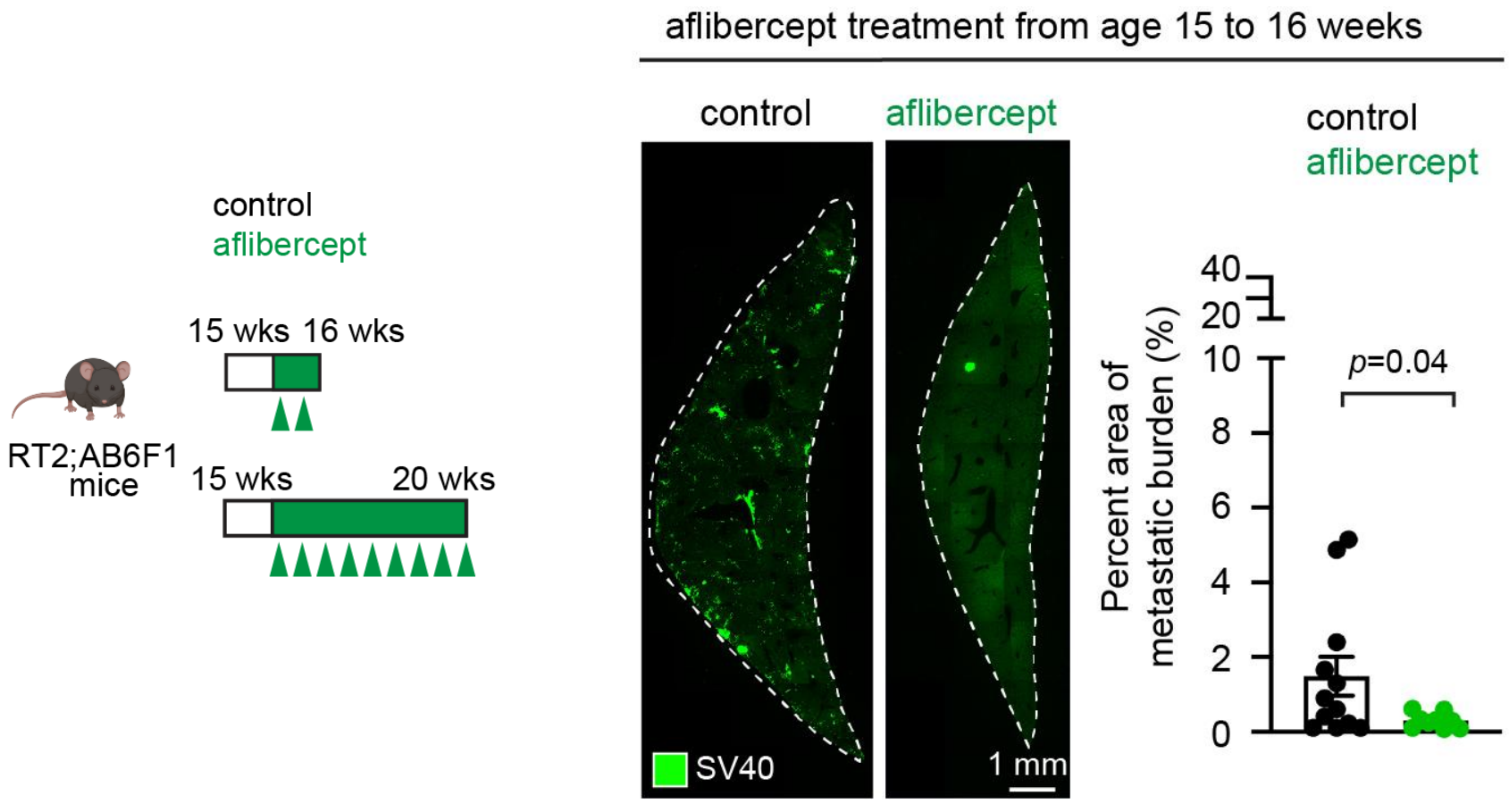
letters to the editor

Reversible tumor growth acceleration following bevacizumab interruption in metastatic colorectal cancer patients scheduled for surgery

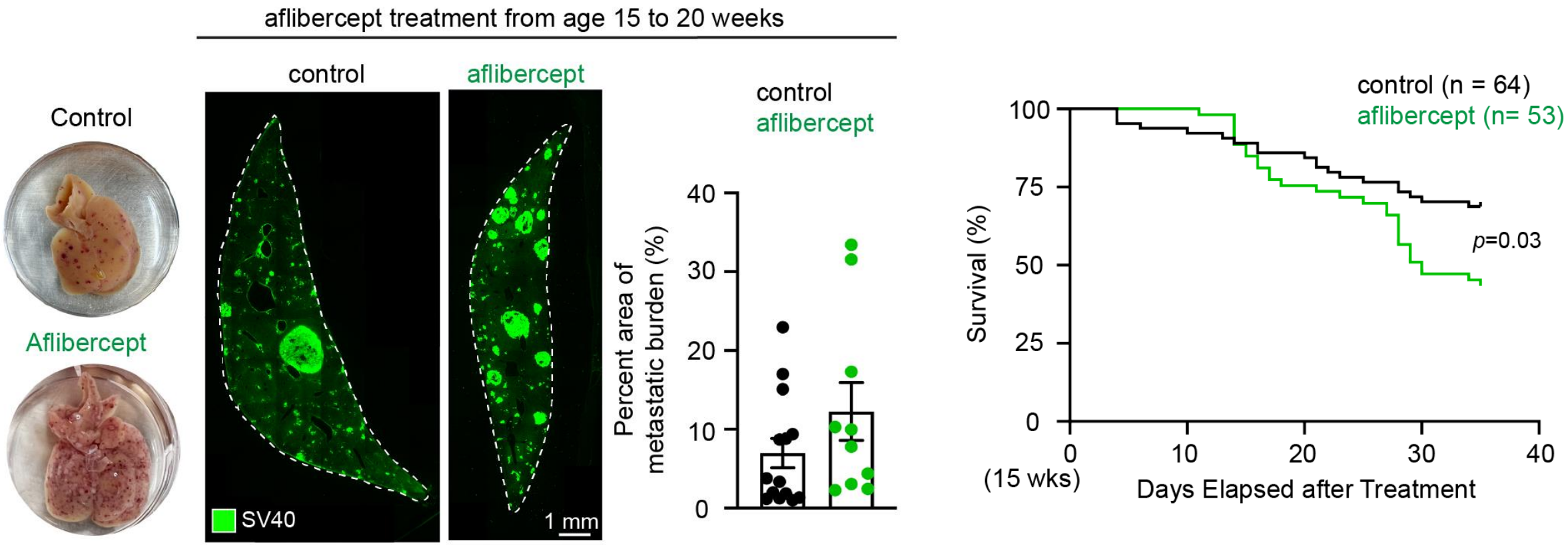
W. Cacheux[1,2], T. Boisserie[2], L. Staudacher[6], O. Vignaux[1,3], B. Dousset[4], O. Soubrane[4], B. Terris[5], C. Mateus[2], S. Chaussade[6] & F. Goldwasser[1,2]*

[1]Angiogenesis Inhibitors Study Group, [2]Department of Medical Oncology, [3]Department of Radiology, [4]Department of Liver Surgery, [5]Department of Pathology, [6]Department of Hepatogastroenterology, Université Paris Descartes, Hôpital Cochin, Assistance Publique-Hôpitaux de Paris, Paris, France
(*E-mail: francois.goldwasser@cch.aphp.fr)

Response to VEGF inhibition in liver metastases of RT2;AB6F1 mice (1-week treatment)



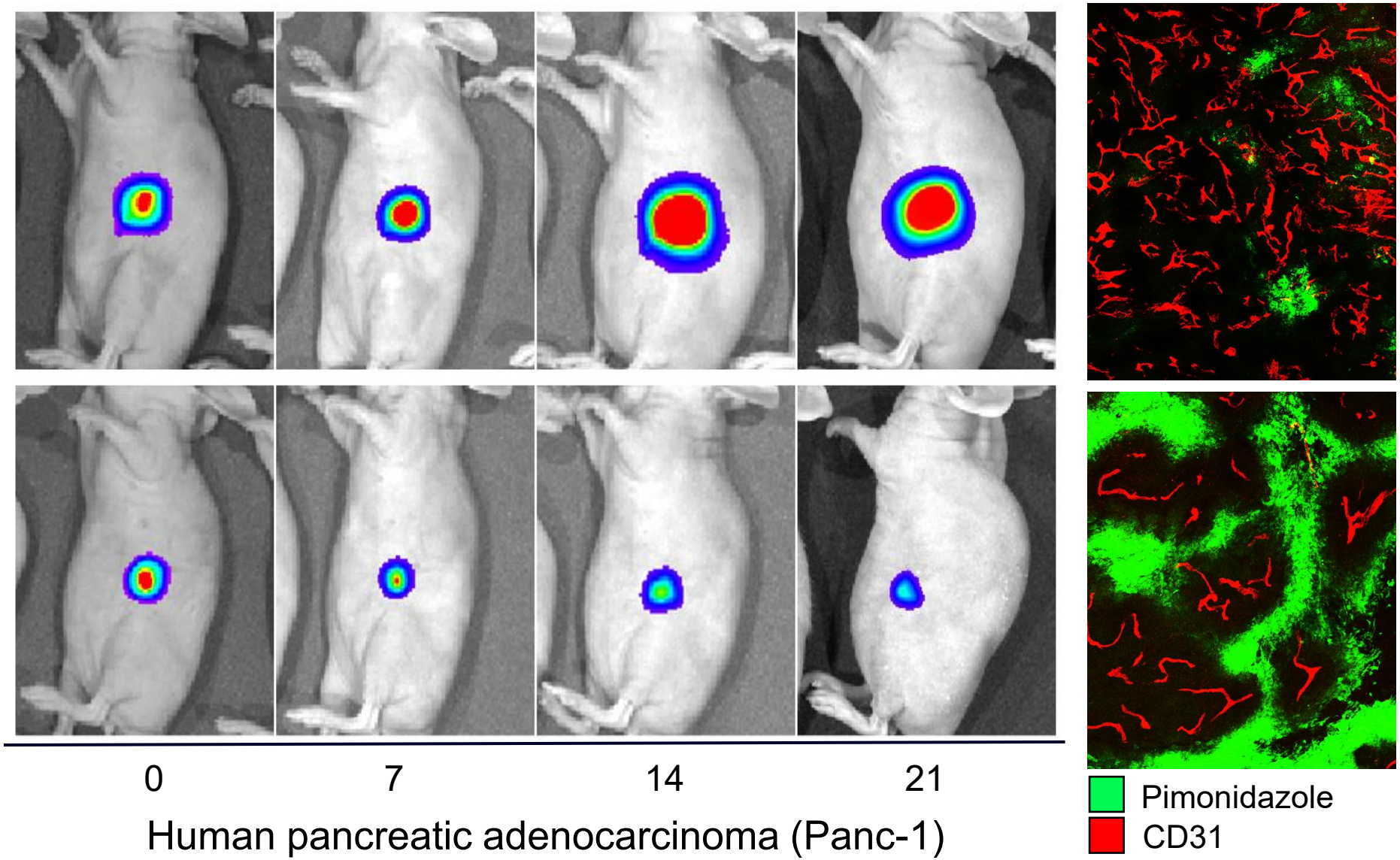
Acquired resistance to VEGF inhibition in liver metastases after prolonged treatment



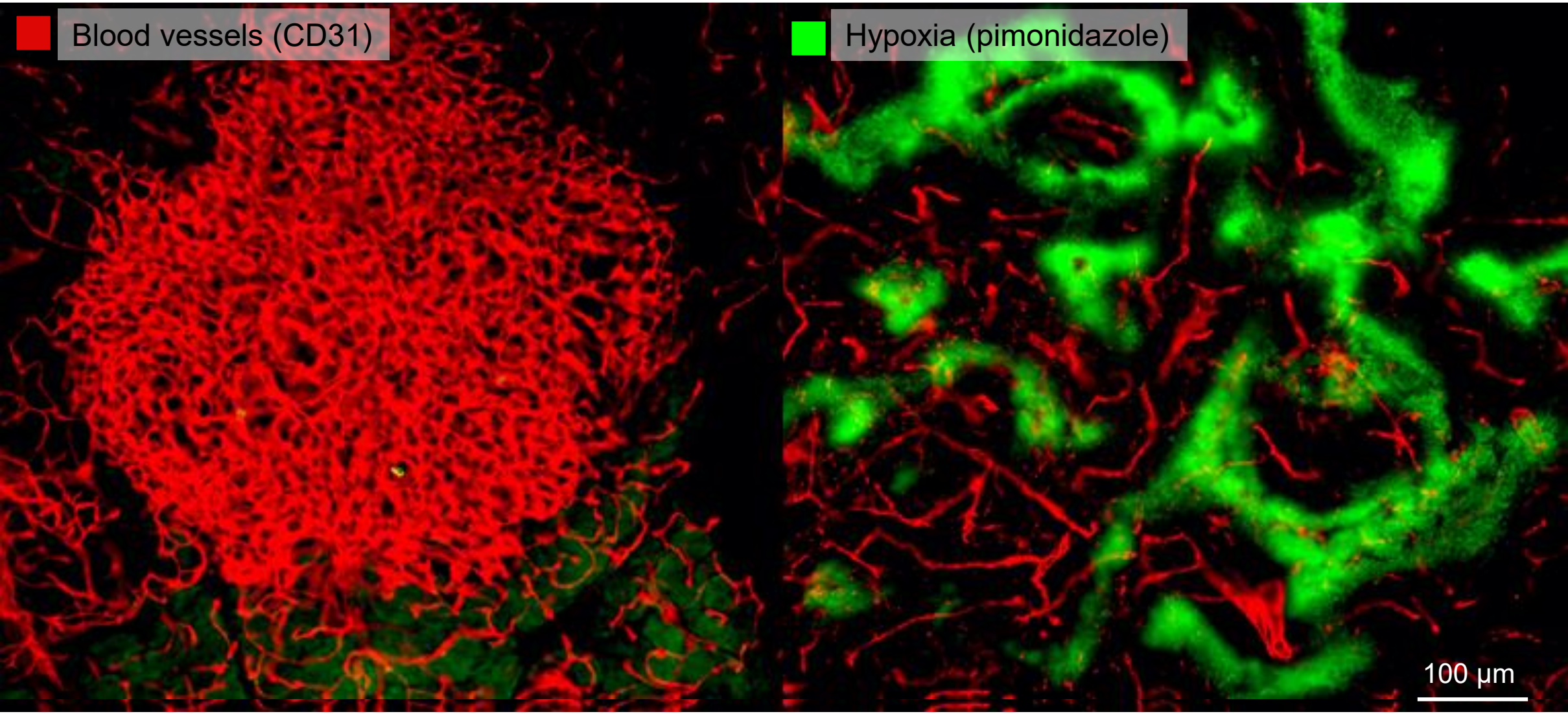
What are the mechanism of resistance to VEGF inhibitor?

1. Other growth factors – from tumor cells or inflammatory cells
2. Tumor cells can co-opt normal blood vessels
3. VEGF-driven vessel regrowth can occur after VEGF inhibition is stopped
4. Intratumoral hypoxia can activate other angiogenic factors that drives invasion and metastasis

Multiple effects of VEGF inhibition: slowing of tumor growth, vascular pruning, and induction of intratumoral hypoxia



Angiogenesis inhibitor actions on pancreatic neuroendocrine tumors: Hypoxia after rapid pruning of tumor vessels



Untreated tumor in RIP-Tag2 mouse

Angiogenesis inhibitor

Effects of angiogenesis inhibitors
: Vascular normalization

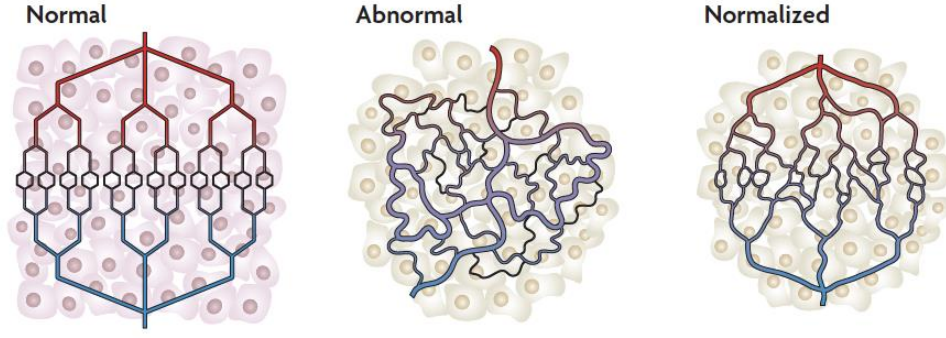
Rakesh Jain

- On blood vessels?

  Stop the growth of new blood vessels

  Destroy some existing blood vessels

  Normalize surviving blood vessels



- On tumor cells?

  Stop tumor growth

  Kill tumor cells by starving the tumor

  ↑ Drug delivery?

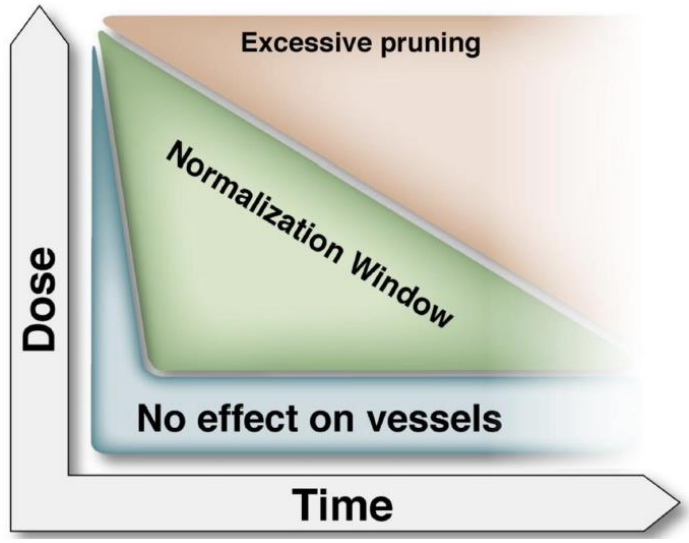
Benefits of vascular normalization from angiogenesis inhibitors are dose- and time-dependent

Vascular normalizing doses of antiangiogenic treatment reprogram the immunosuppressive tumor microenvironment and enhance immunotherapy

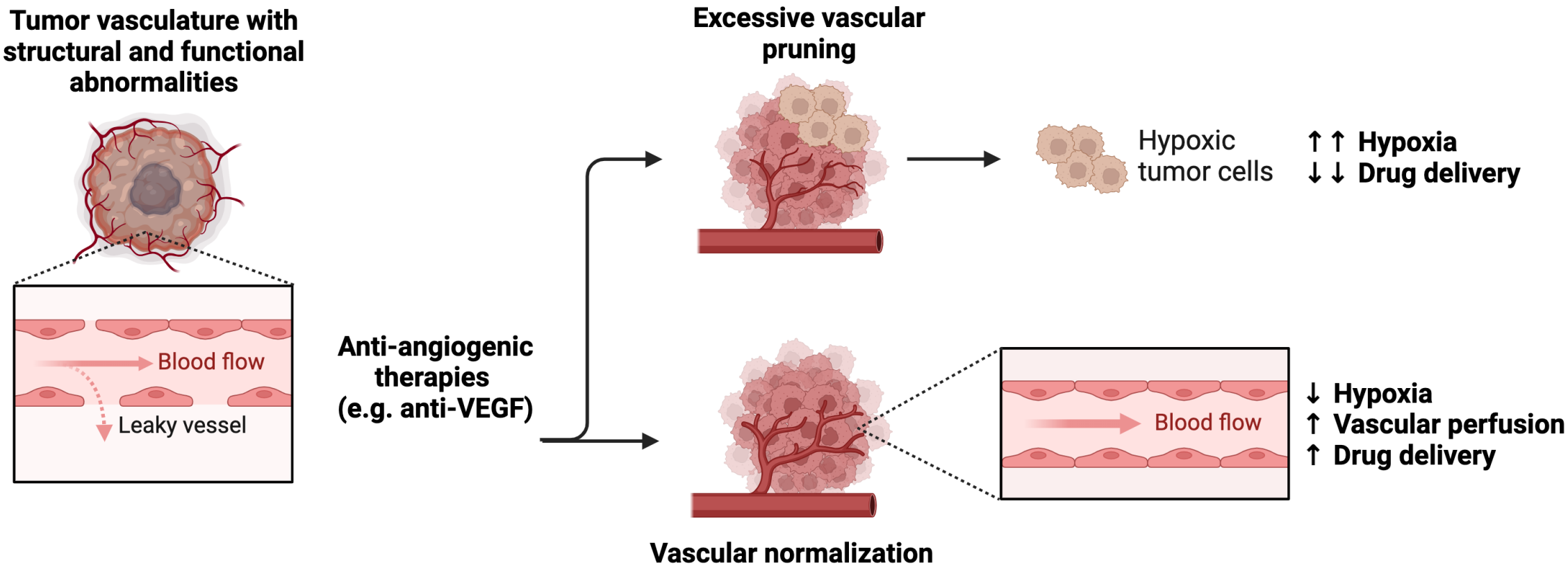
Yuhui Huang[a], Jianping Yuan[b], Elda Righi[b], Walid S. Kamoun[a], Marek Ancukiewicz[a], Jean Nezivar[b], Michael Santosuosso[b], John D. Martin[a], Margaret R. Martin[a], Fabrizio Vianello[b], Pierre Leblanc[b], Lance L. Munn[a], Peigen Huang[a], Dan G. Duda[a], Dai Fukumura[a], Rakesh K. Jain[a,1], and Mark C. Poznansky[b]

PNAS 2010

Normalization window is dose- and time-dependent

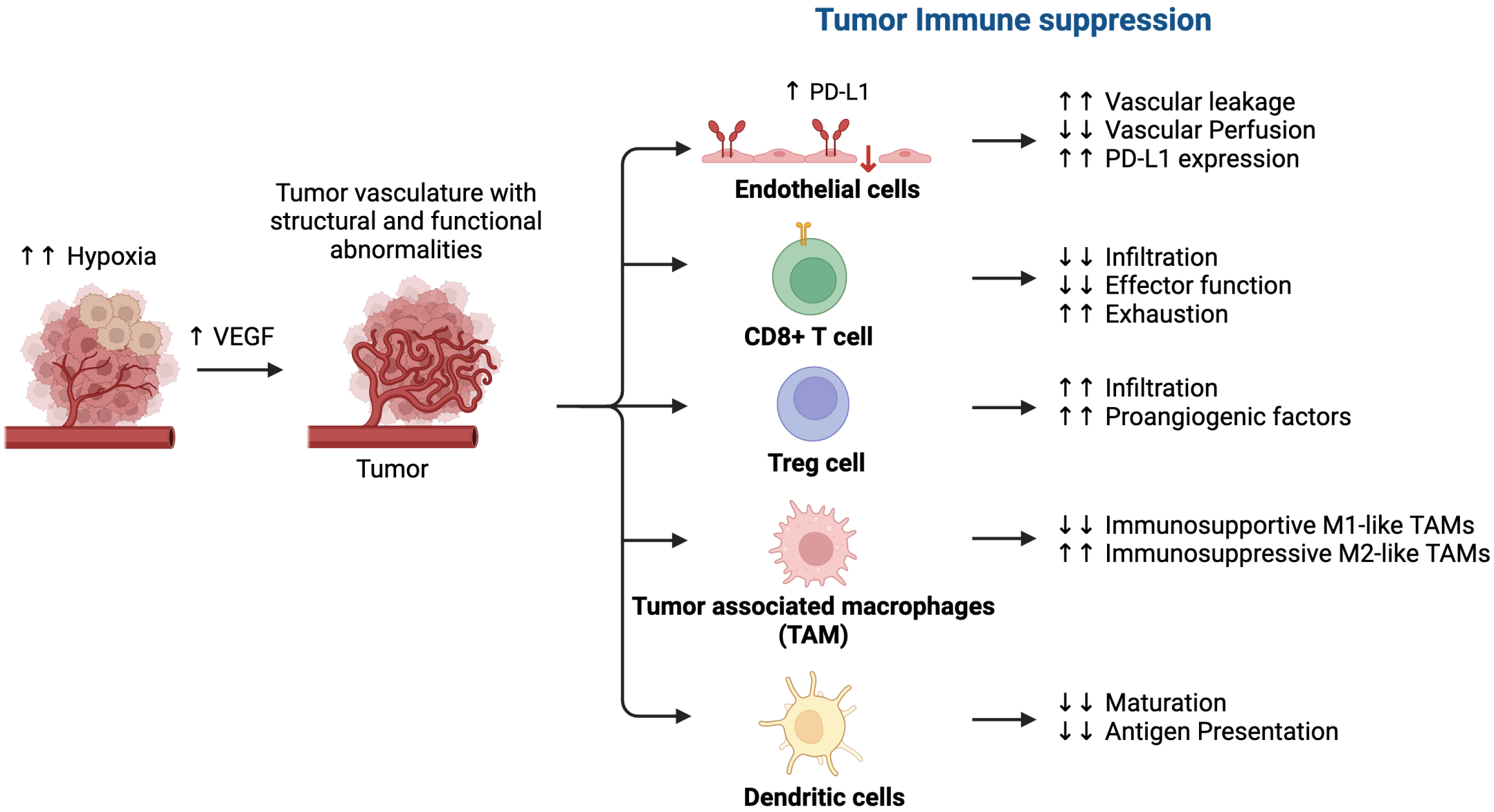


Targeting the tumor vasculature in cancer treatment



Tong *et al*. Caner Res 64: 3731-36, 2004
Rakesh Jain Science 307: 58-62, 2005

Tumor vascular abnormalities induce immune suppression



Targeting the tumor vasculature to promote immune activation and increase immunotherapy efficacy

Atezolizumab plus Bevacizumab in Unresectable Hepatocellular Carcinoma

Finn et al., NEJM. 2020

Atezolizumab plus bevacizumab versus sunitinib in patients with previously untreated metastatic renal cell carcinoma (IMmotion151): a multicentre, open-label, phase 3, randomised controlled trial

Rini et al., Lancet. 2019

OPINION

Improving immune–vascular crosstalk for cancer immunotherapy

Yuhui Huang, Betty Y. S. Kim, Charles K. Chan, Stephen M. Hahn, Irving L. Weissman and Wen Jiang

Nat Rev Immunol. 2018

Improving immunotherapy outcomes with anti-angiogenic treatments and vice versa

Kabir A. Khan and Robert S. Kerbel**

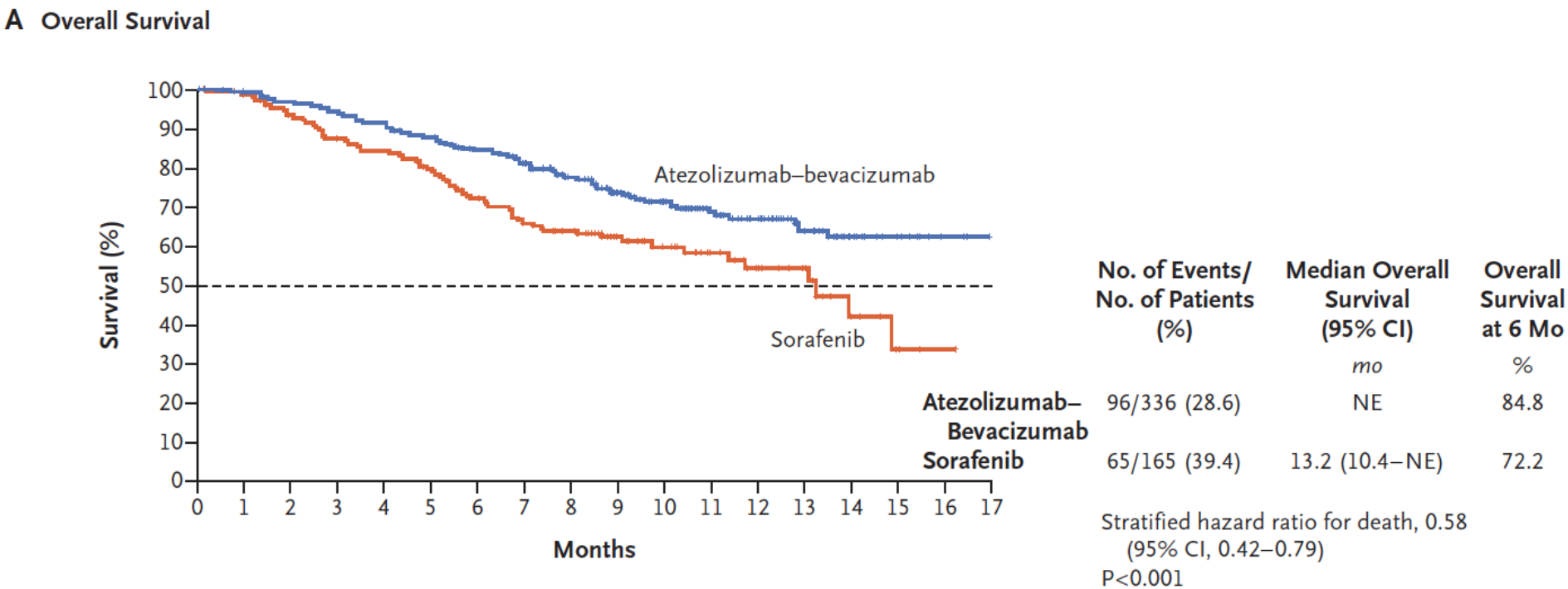
Nat Rev Calin Oncol. 2018

Enhancing cancer immunotherapy using antiangiogenics: opportunities and challenges

Dai Fukumura, Jonas Kloepper, Zohreh Amoozgar, Dan G. Duda and Rakesh K. Jain

Nat Rev Clin Oncol. 2018

Anti-PD-L1 plus anti-VEGF in unresectable HCC



	No. of Events/ No. of Patients (%)	Median Overall Survival (95% CI)	Overall Survival at 6 Mo
		mo	%
Atezolizumab–Bevacizumab	96/336 (28.6)	NE	84.8
Sorafenib	65/165 (39.4)	13.2 (10.4–NE)	72.2

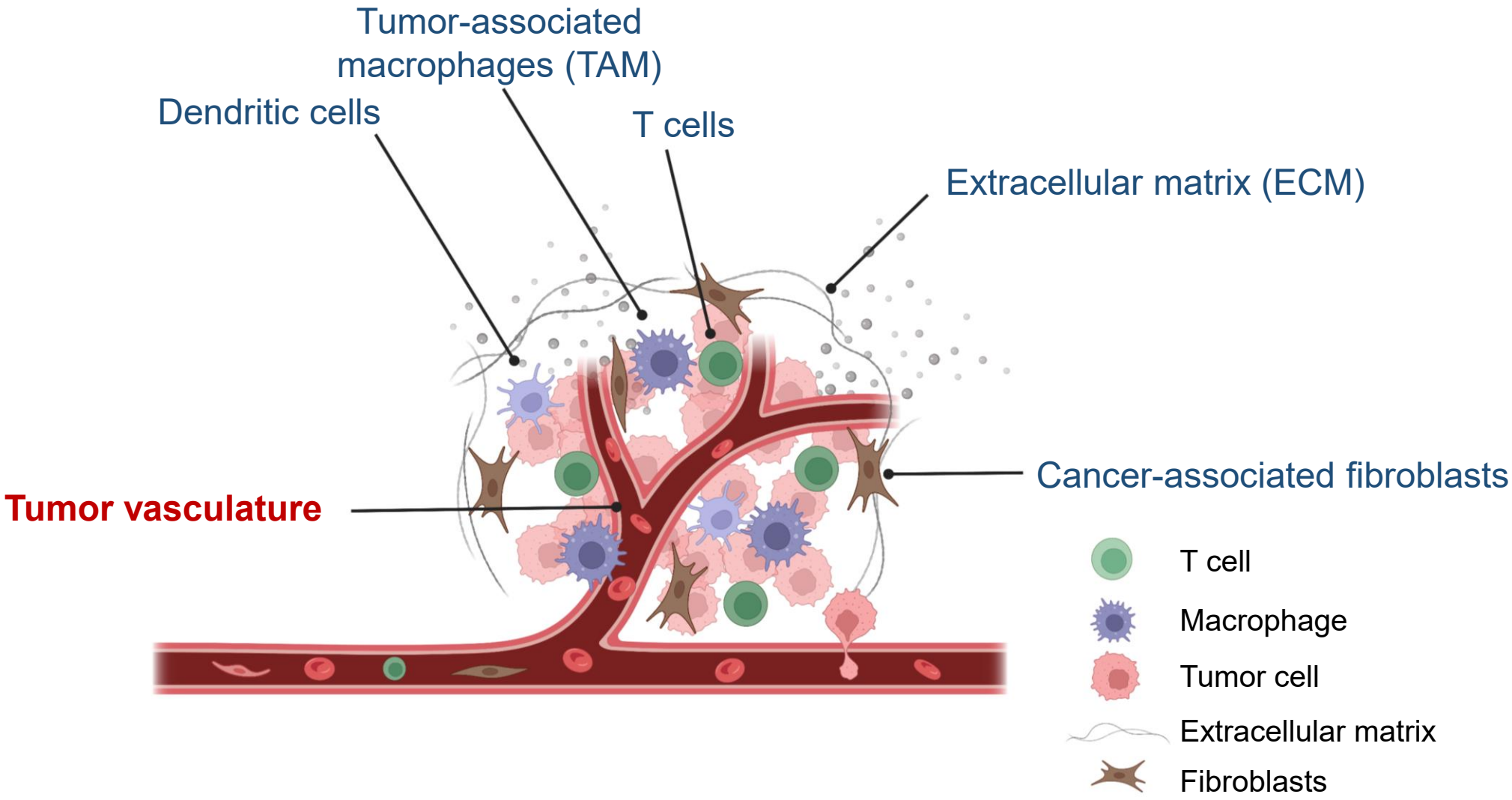
Stratified hazard ratio for death, 0.58 (95% CI, 0.42–0.79)
P<0.001

Finn., N Engl J Med 2020

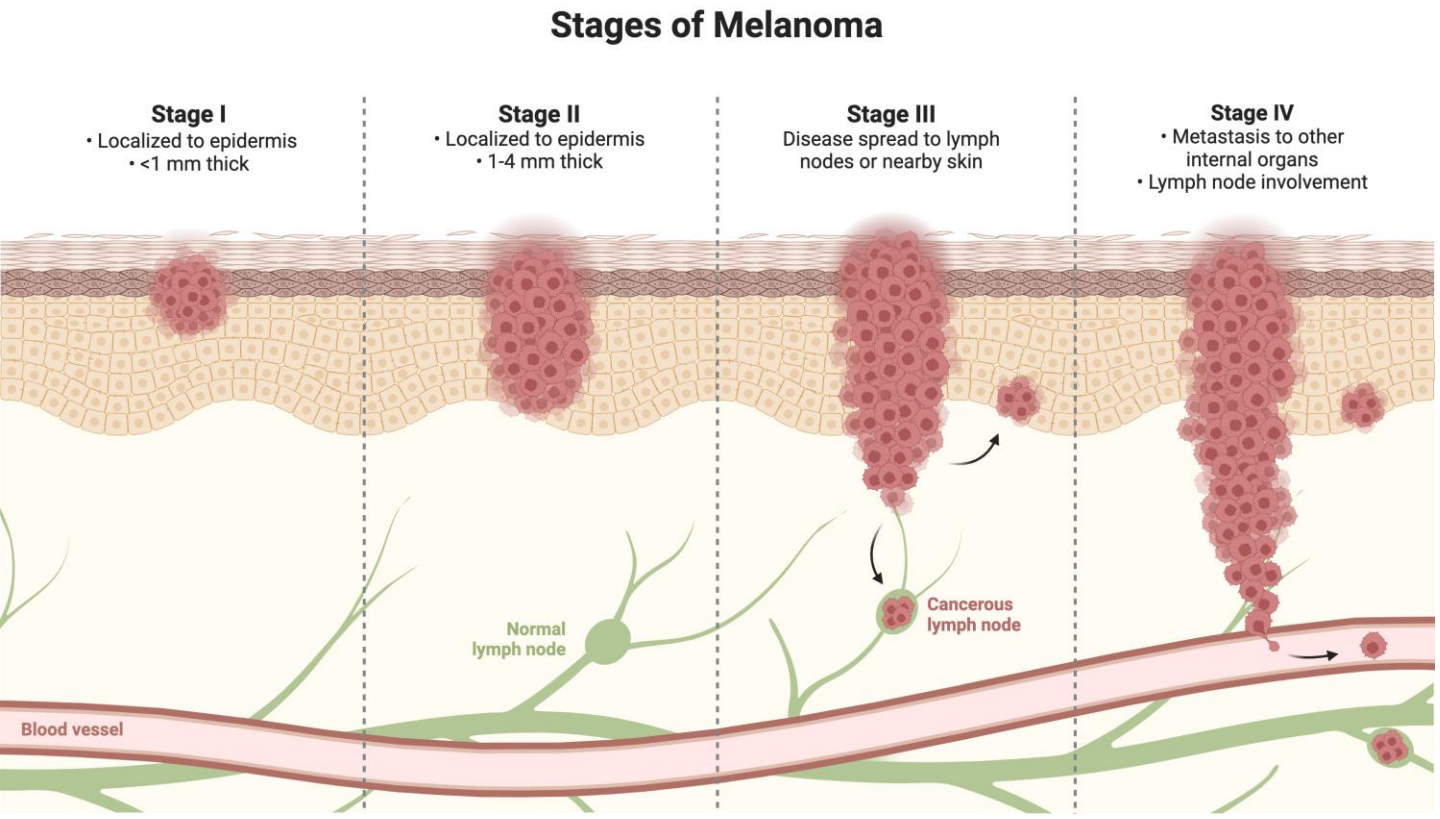
Current clinical studies testing combinations of antiangiogenic agents and immune checkpoint inhibitors

Immune-checkpoint blockade studies			
Ipilimumab (anti-CTLA-4 mAb; 3 or 10 mg/kg)	Bevacizumab (7.5 or 15 mg/kg)	Advanced-stage melanoma	• Phase I: 8 of 46 patients had a PR, and 22 had SD • Increased numbers of tumour $CD8^+$ T cells and $CD163^+$ dendritic macrophages, and increased numbers of circulating memory T cells with combination versus ipilimumab alone
Durvalumab (anti-PD-L1 mAb)	Bevacizumab	Glioblastoma	Phase II: active, not recruiting (PFS, OS, and AEs)
Pembrolizumab (anti-PD-1 mAb)	Bevacizumab	Glioblastoma	Phase II: active, not recruiting (RP2D and/or MTD of combination, and PFS)
Nivolumab (anti-PD-1 mAb)	Bevacizumab	NSCLC	Phase I: active, not recruiting (safety and tolerability, ORR, and RFS)
Ipilimumab or nivolumab	Bevacizumab (combined with ipilimumab only)	Metastatic melanoma	• Phase NA: complete • High pre-treatment serum ANG2 associated with worse OS • Ipilimumab increased serum ANG2 levels and ipilimumab plus bevacizumab decreased serum ANG2 levels
MOXR0916 (agonistic anti-TNFRSF4 mAb) + atezolizumab (anti-PD-L1 mAb)	Bevacizumab	Advanced-stage solid tumours	Phase I: active, not recruiting (AEs, DLTs, and ORR)
Tremelimumab (anti-CTLA-4 mAb) or durvalumab	Bevacizumab	Resectable CRC liver metastases	Phase I: recruiting (feasibility and RFS)
Ipilimumab	Cabozantinib (VEGFR TKI)	Metastatic genitourinary tumours	Phase I: recruiting (AEs, RP2D, ORR, and PD-L1 and MET expression)
Atezolizumab (anti-PD-L1 mAb)	Vanucizumab (bi-specific mAb targeting VEGF and ANG2)	Advanced-stage solid tumours	Phase I: active, not recruiting (MTD, AEs, and ORR)
RO7009789 (agonistic anti-CD40 mAb)	Vanucizumab	Metastatic solid tumours	Phase I: recruiting (safety, pharmacokinetics and pharmacodynamics, and therapeutic activity)

The mechanistic contribution of antiangiogenic therapy to clinical response when combined with immunotherapy remains elusive.



Melanoma : highly aggressive cancer

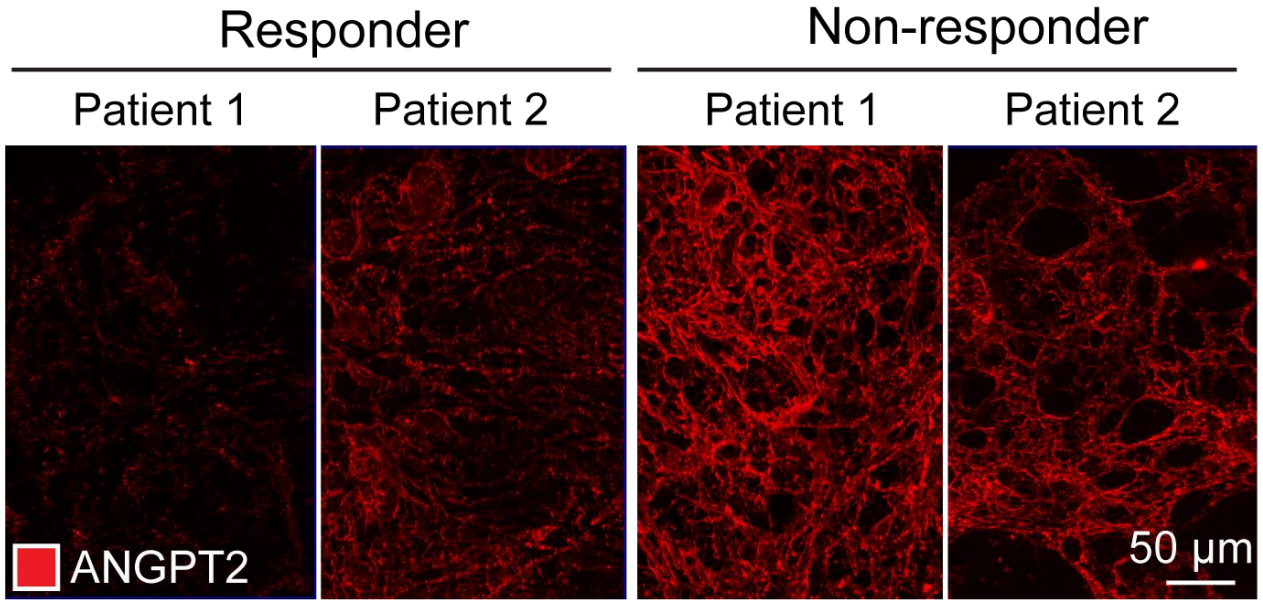


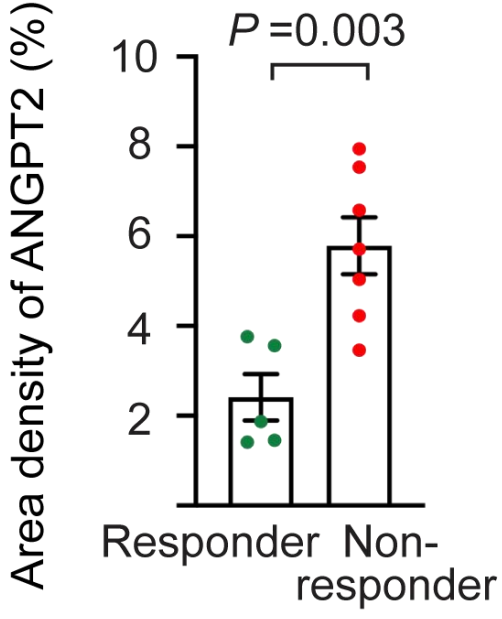
Response to immune checkpoint inhibitor therapies

- Anti-PD-1: 5-year overall survival of 44%
- Anti-PD-1 + anti-CTLA-4: 5-year overall survival of 52%
- Primary resistance in 30-50% of patients and secondary resistance in 20-30%

Atkins et al., Clinical Cancer Res, 2021

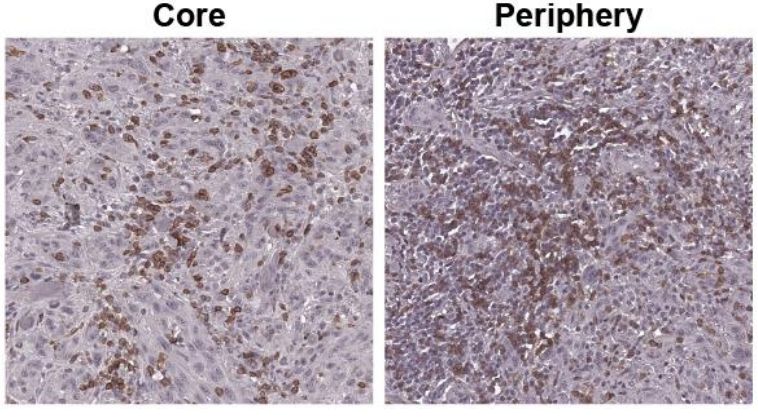
High pretreament ANGPT2 expression associates with resistance to PD-1 blockade in melanoma patients



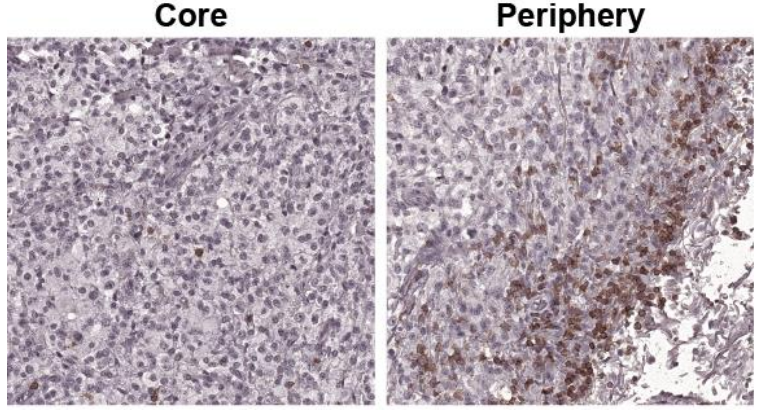


T-cell exclusion: a mechanism of immune evasion and resistance to immunotherapy

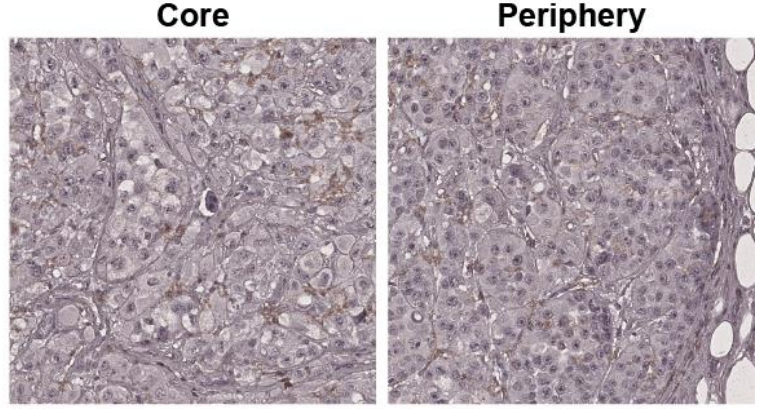
Inflamed
Immune cells infiltrated



Immune excluded
CD8+ T cells accumulated but have not efficiently infiltrated

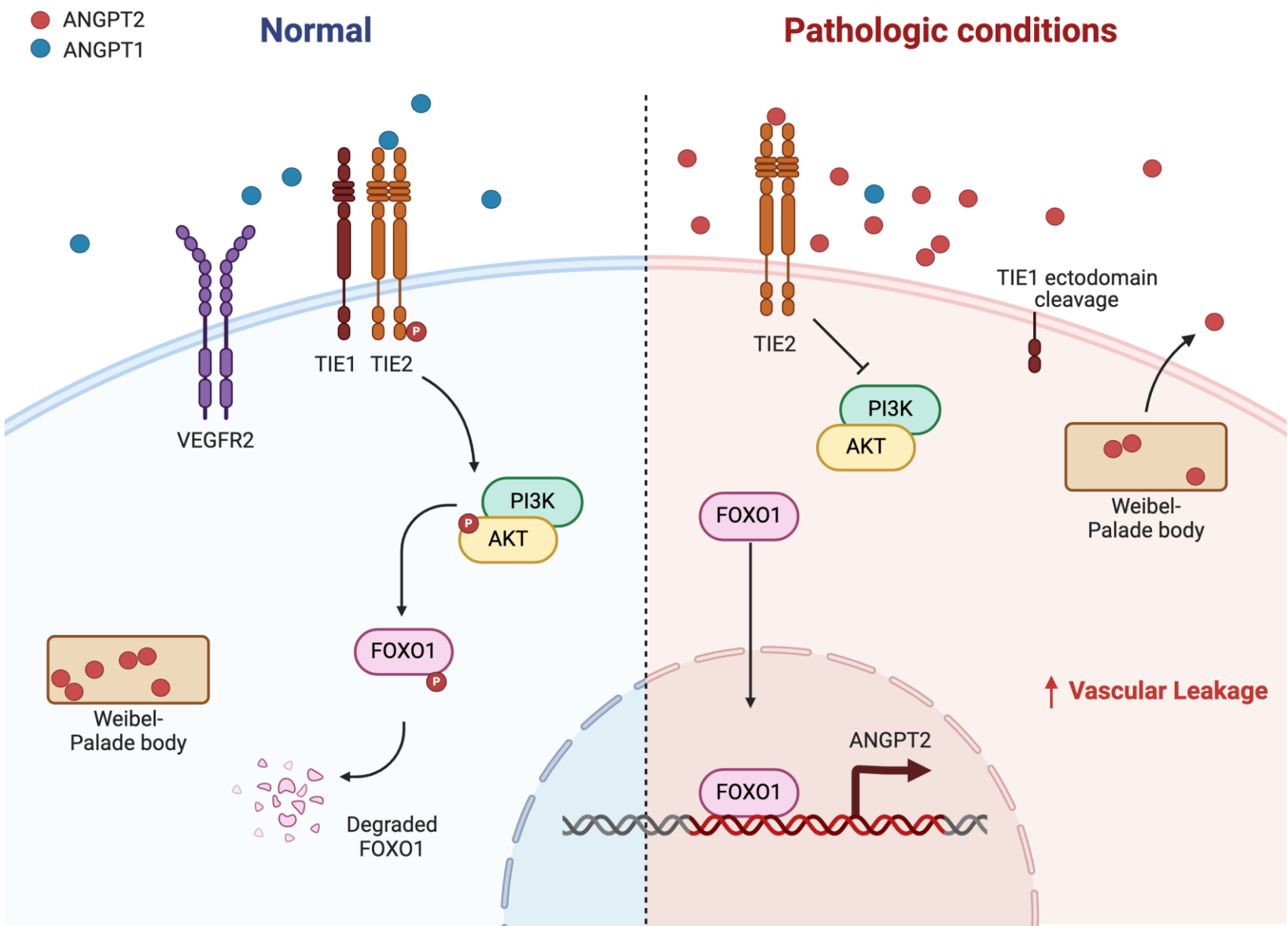


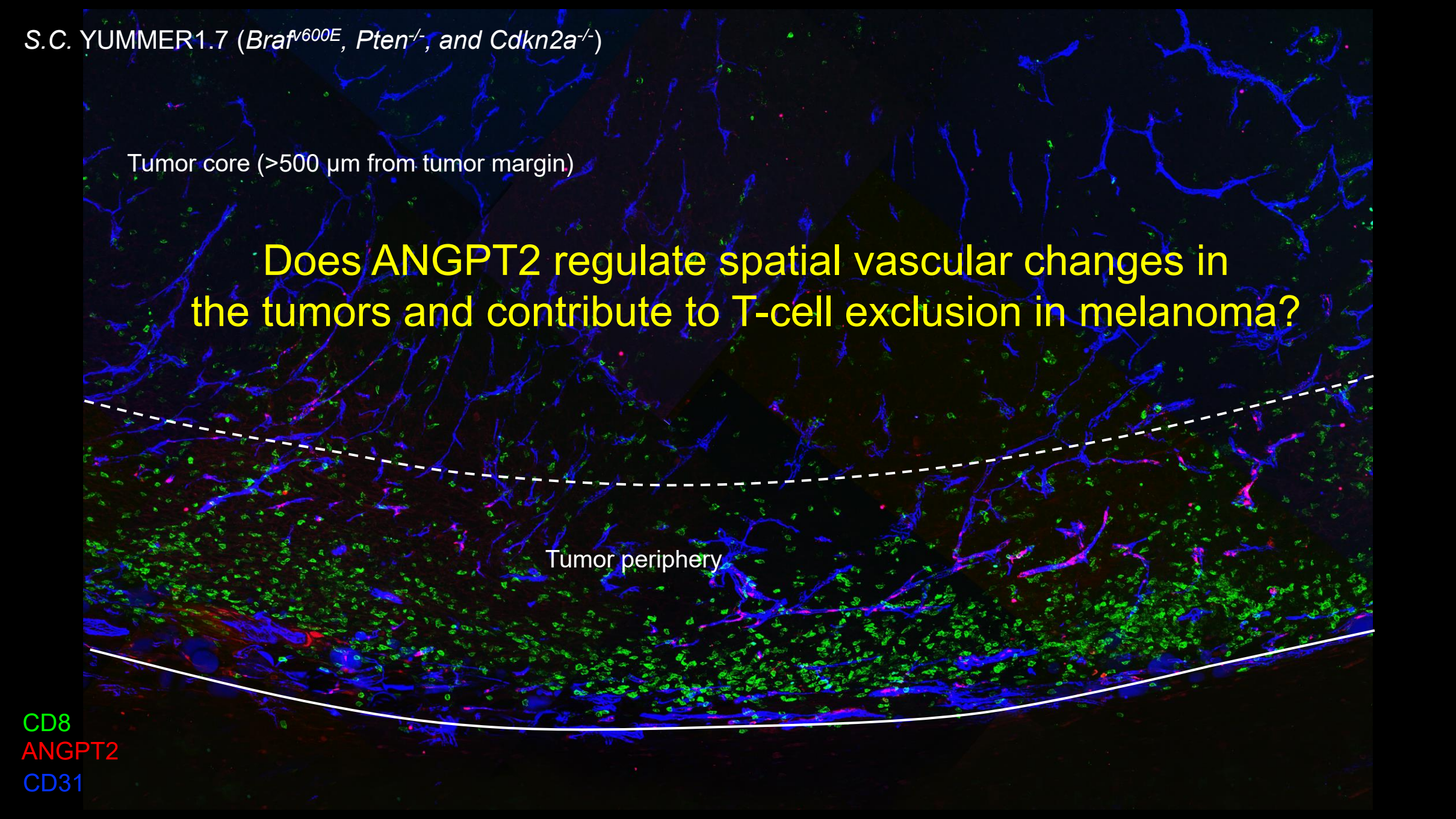
Immune desert
CD8+ T cells absent from tumor and its periphery



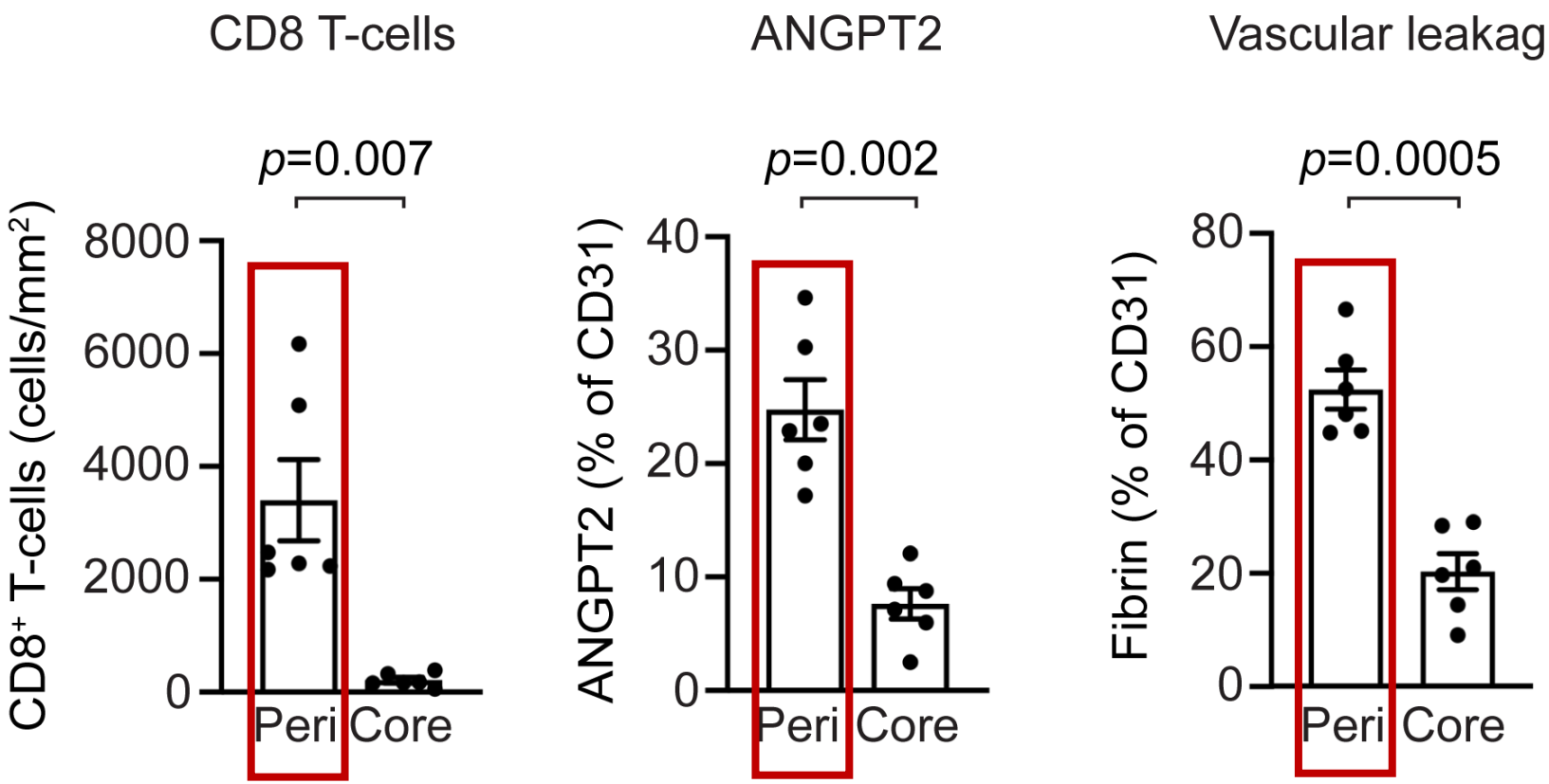
CD8

Angiopoietin-2 (ANGPT2)/TIE2 signaling pathway




S.C. YUMMER1.7 (Braf^{V600E}, Pten$^{-/-}$, and Cdkn2a$^{-/-}$)
Tumor core (>500 μm from tumor margin)
Does ANGPT2 regulate spatial vascular changes in the tumors and contribute to T-cell exclusion in melanoma?
Tumor periphery
CD8
ANGPT2
CD31

ANGPT2 upregulation and increased vascular leakage in the tumor periphery



Functional studies for ANGPT2 in mice

YUMM1.7

- *Braf^{V600E}, Pten$^{-/-}$, and Cdkn2a$^{-/-}$*
- Resistant to checkpoint blockade

YUMMER1.7 (YUMM Exposed to Radiation)

- Braf^{V600E}, Pten$^{-/-}$, and Cdkn2a$^{-/-}$
- Additional somatic mutation by UV exposure
- Responsive to checkpoint blockade

Collaboration with Dr. Amanda Lund at NYU

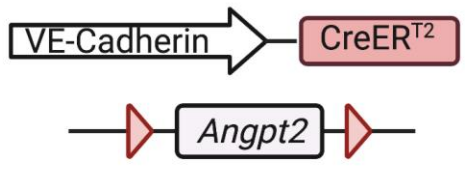
Pharmacological Inhibition

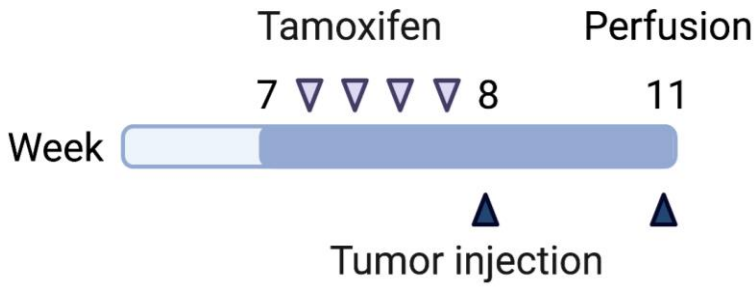
C57BL/6 mice

IgG or
anti-ANGPT2 (REGN910)

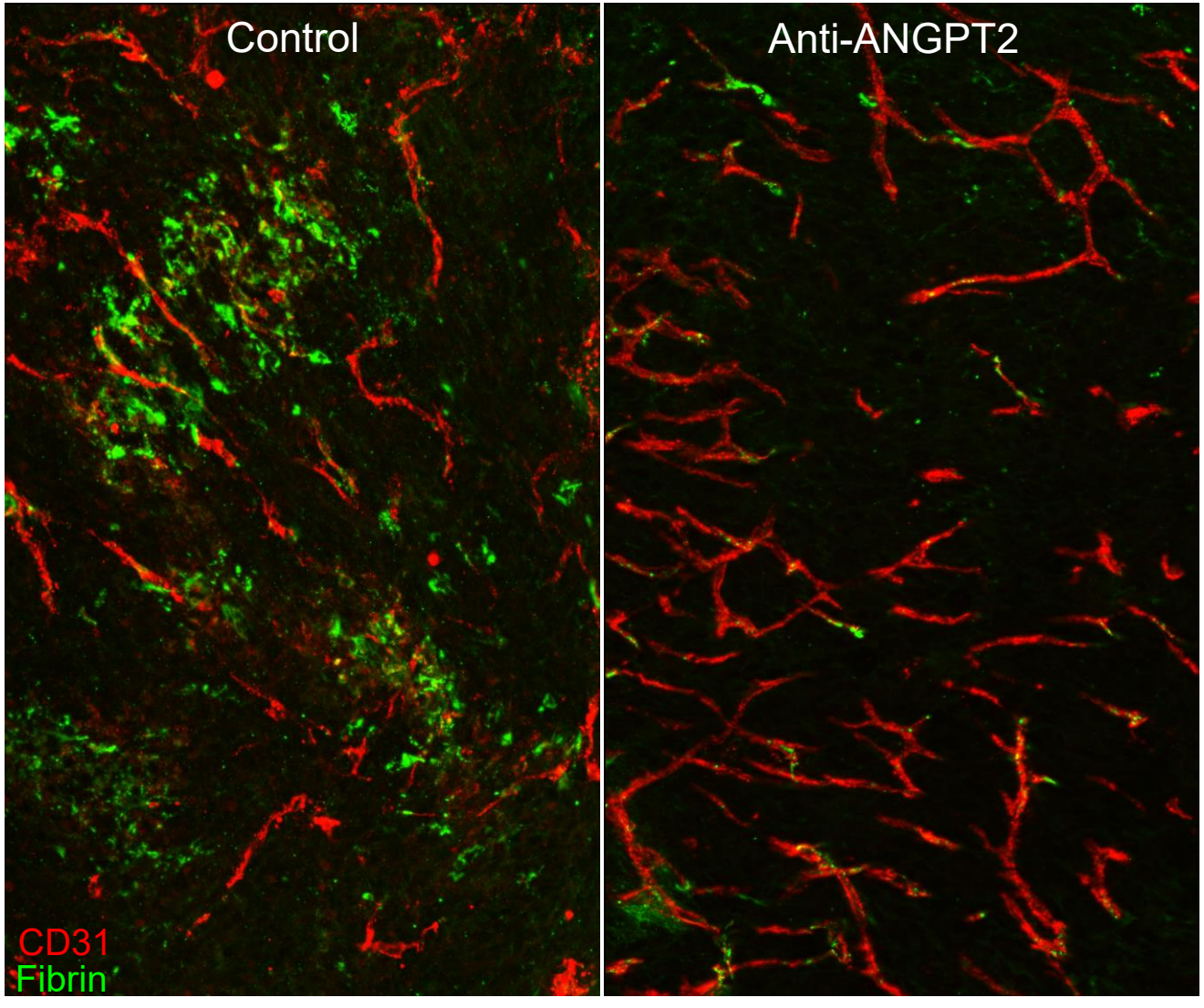
Genetic Deletion

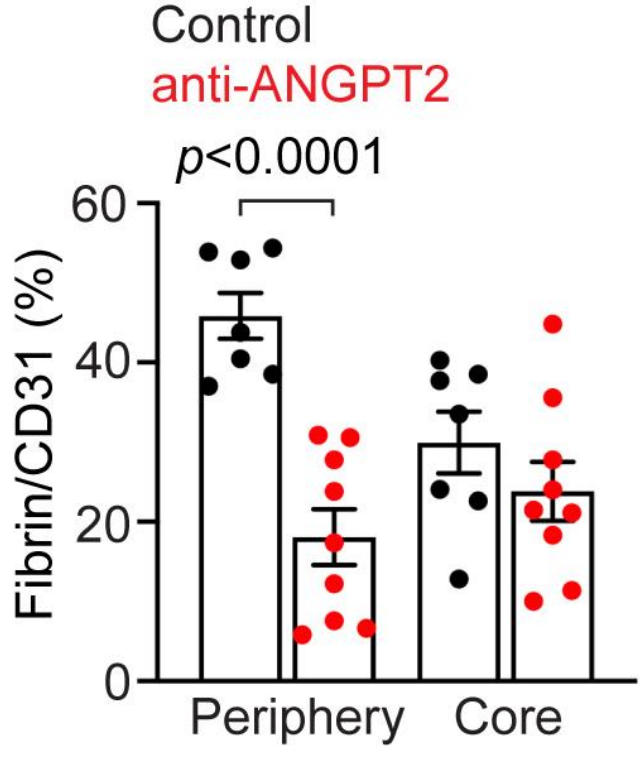
Angpt2$^{i\Delta EC}$

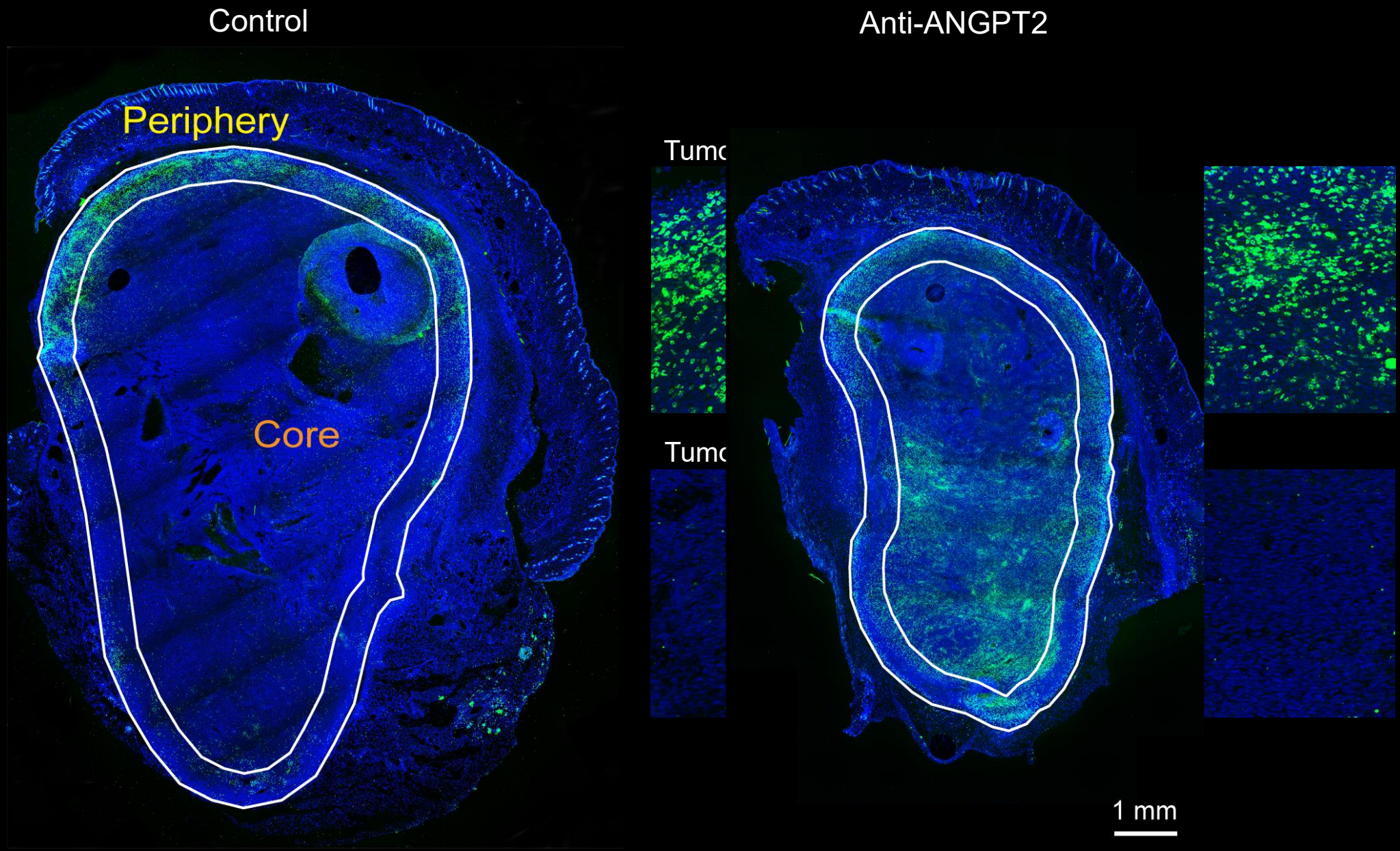




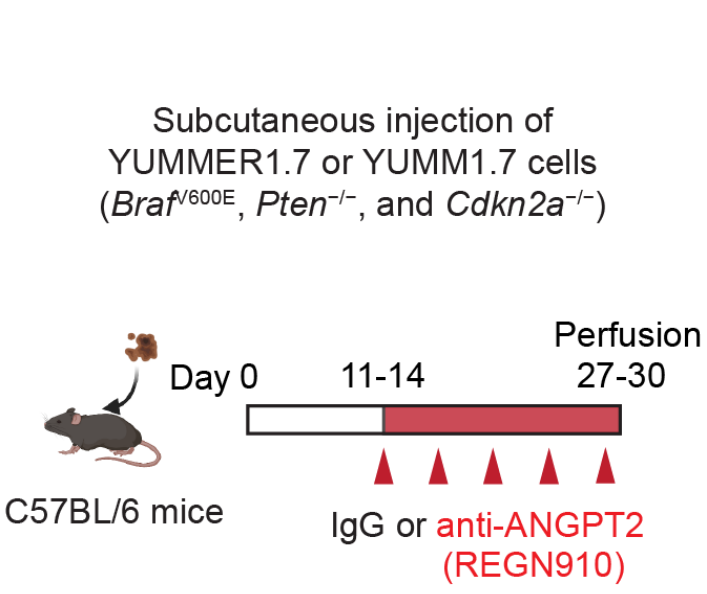
Reduced vascular leakage at the tumor periphery after ANGPT2 inhibition in melanoma

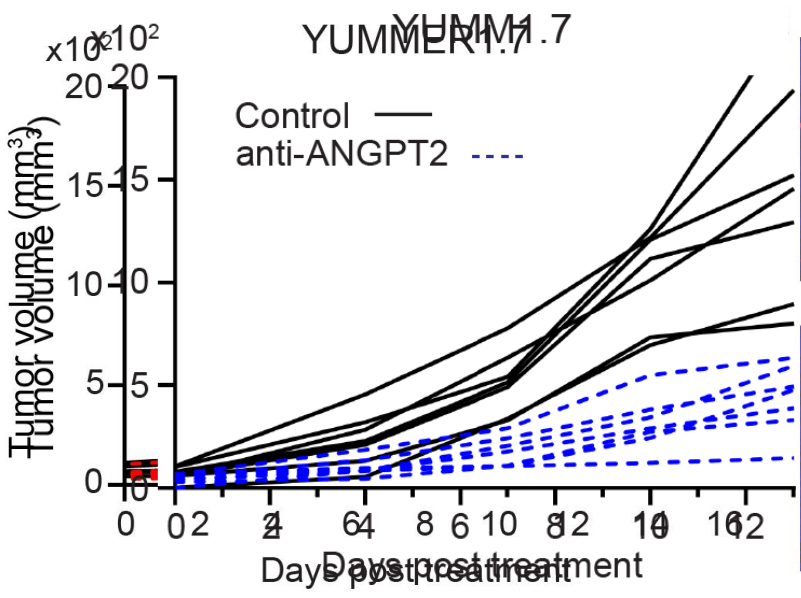




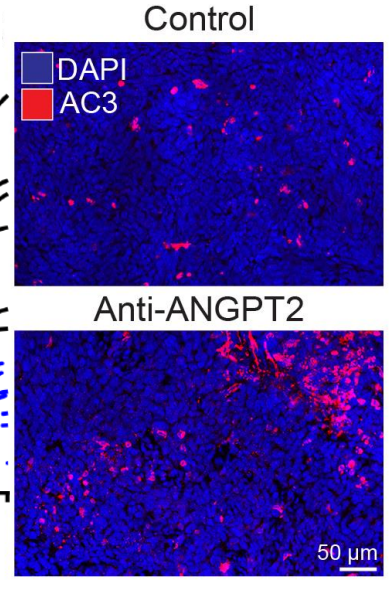

Control
Anti-ANGPT2
Periphery
Core
Tumo
Tumo
1 mm

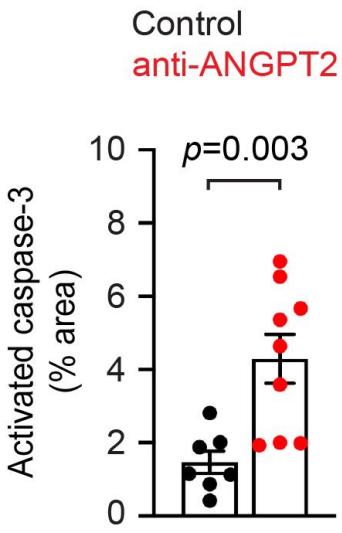
ANGPT2 blockade exhibits significant anti-tumor efficacy in melanoma



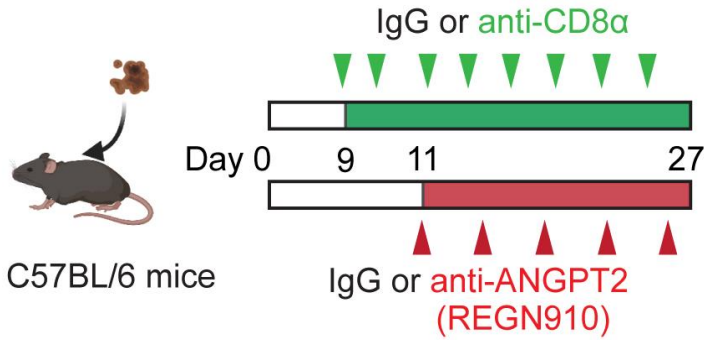


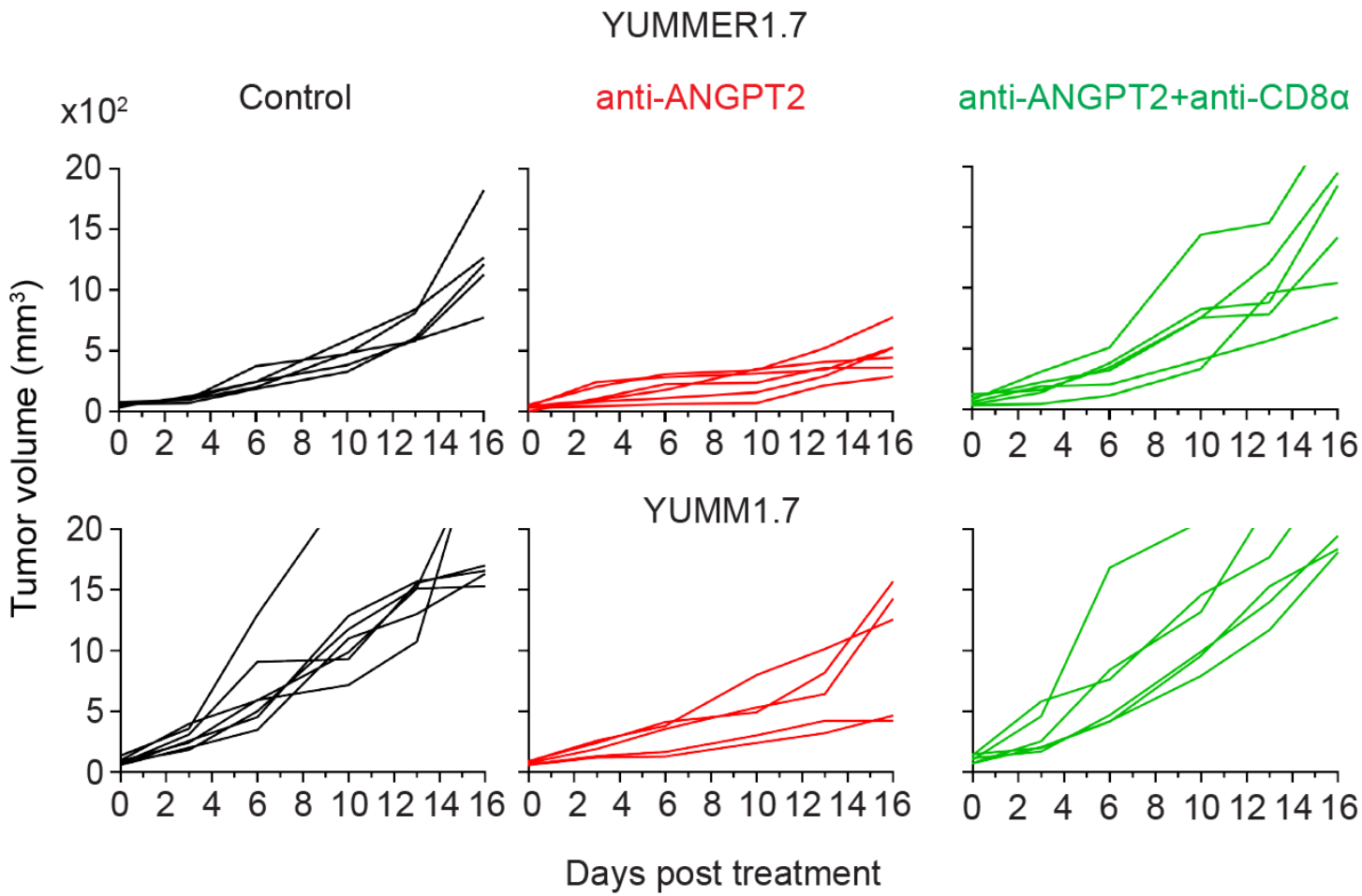
Tumor apoptosis





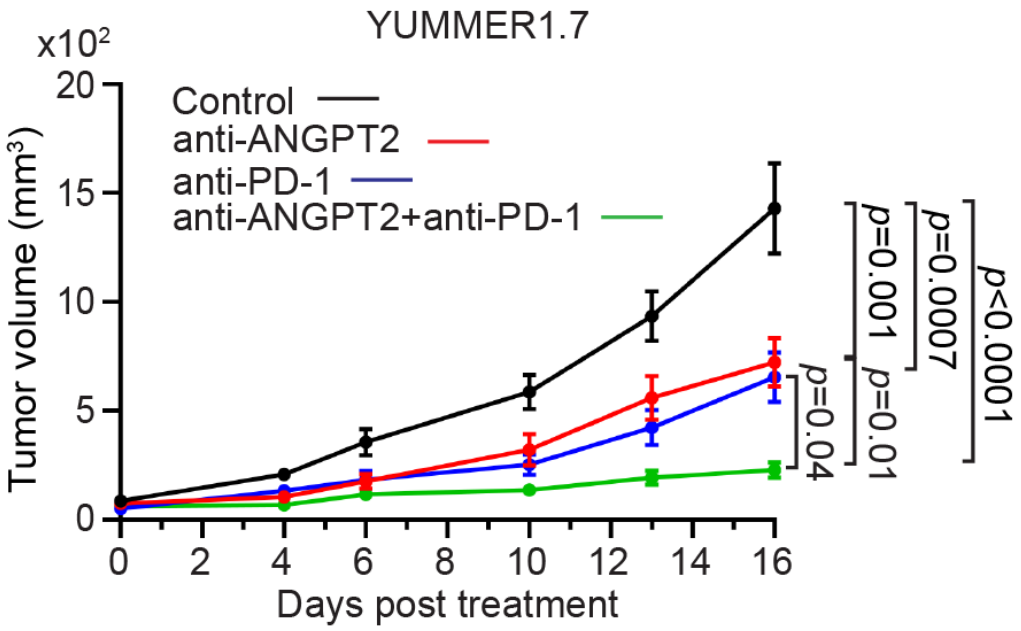
CD8⁺ T cells mediate anti-tumor response to ANGPT2 inhibition



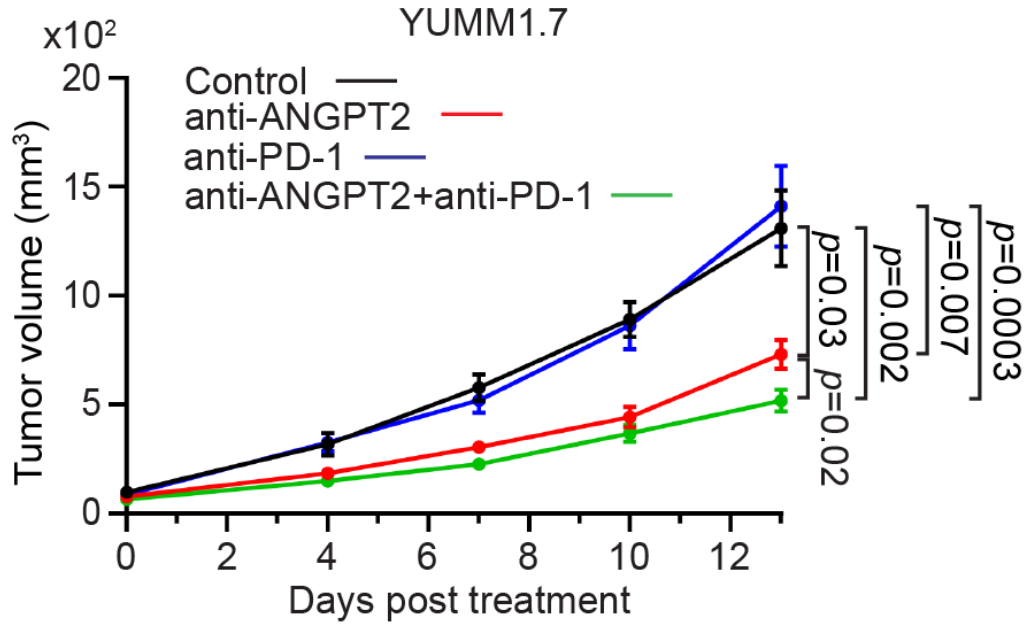


ANGPT2 inhibition enhances anti-tumor efficacy of anti-PD-1 therapy

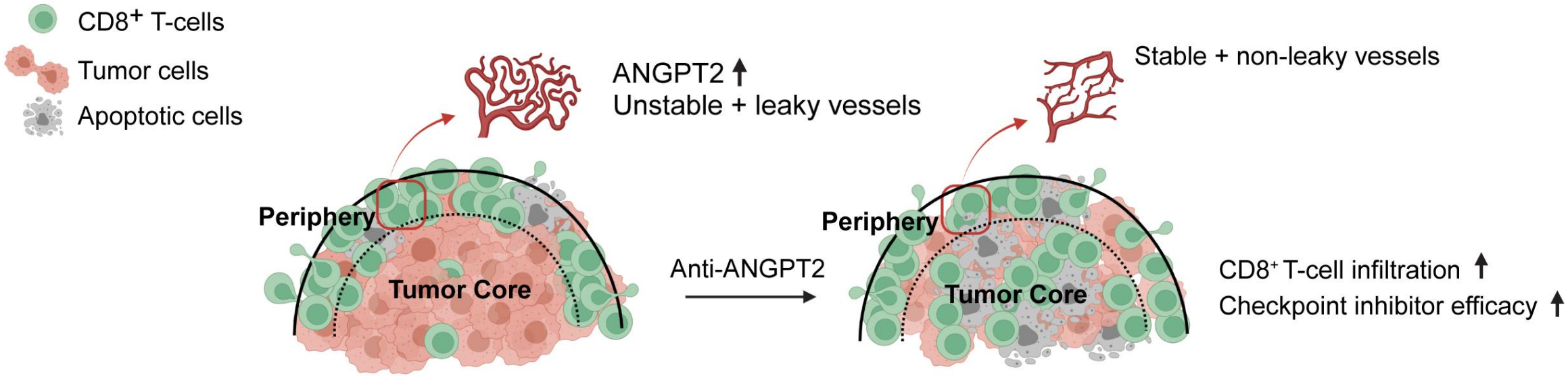
YUMMER1.7 (responsive to anti-PD-1)



YUMM1.7 (resistant to anti-PD-1)

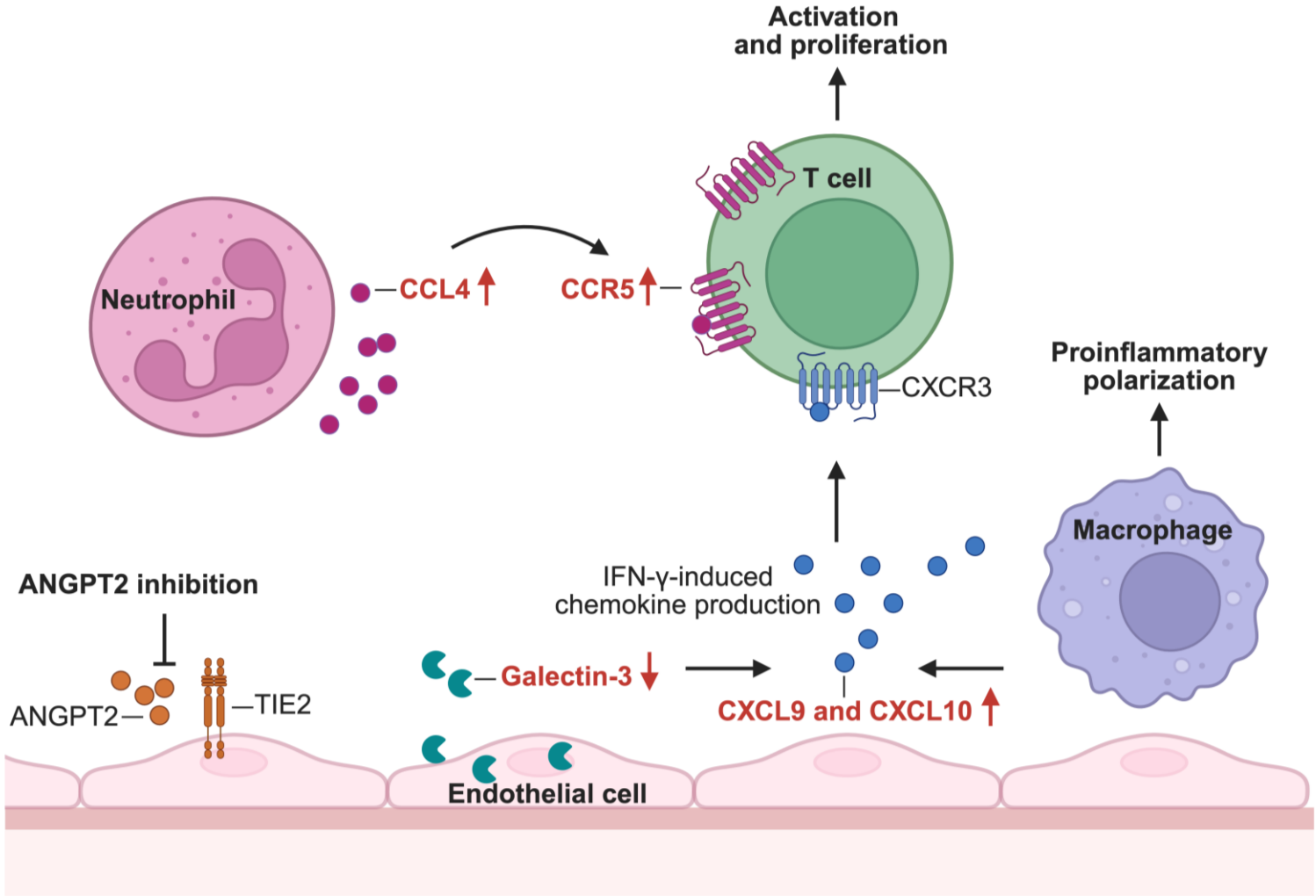


Angiopoietin-2-dependent spatial vascular destabilization promotes T-cell exclusion and limits immunotherapy in melanoma



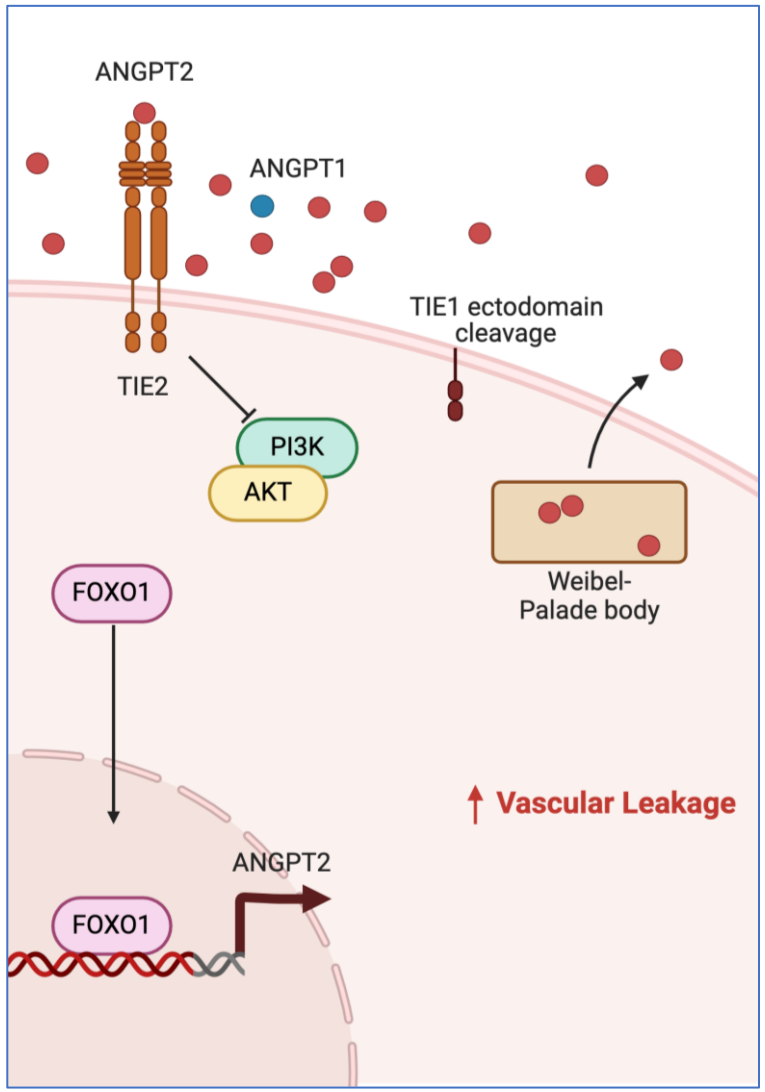
Park et al., Caner Res. 2023

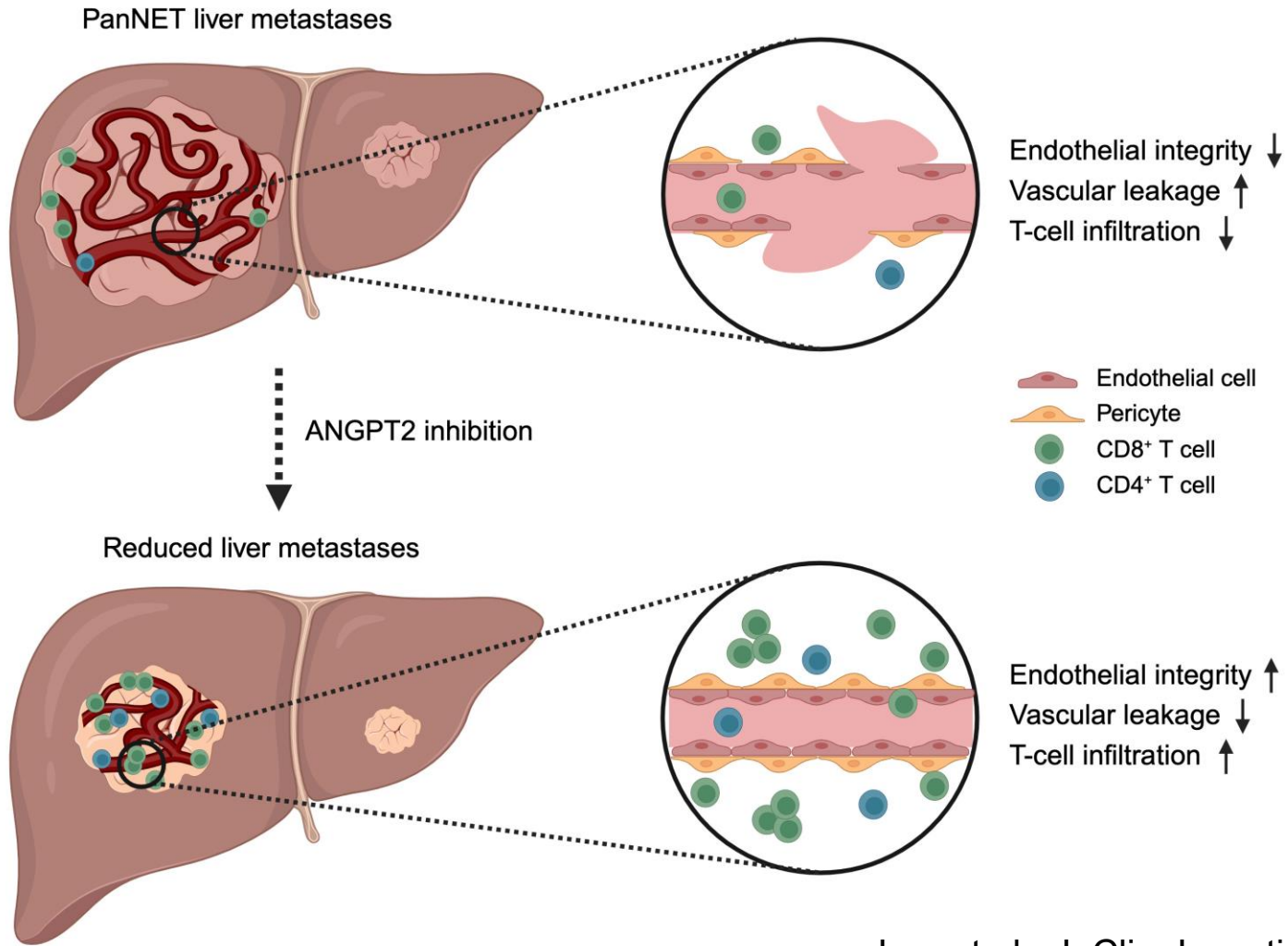
Mechanistic insights into tumor immune activation mediated by ANGPT2 inhibition



Unpublished

ANGPT2 blockade suppresses growth of liver metastases from PanNET by promoting T cell recruitment





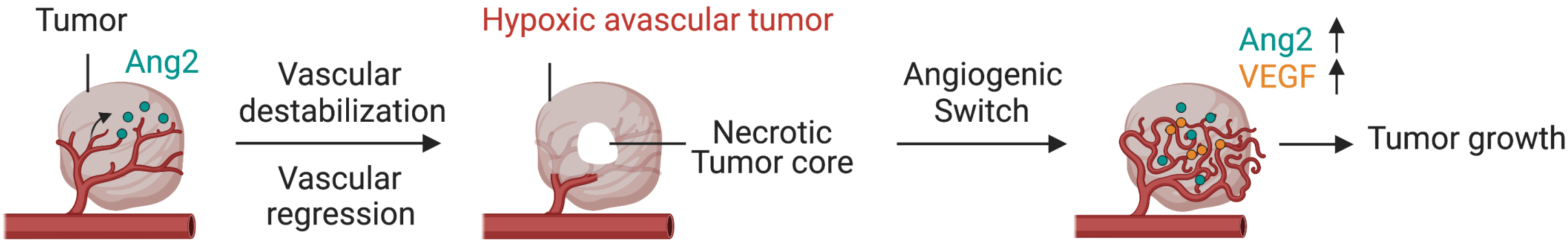
Lee et al., J. Clin. Investig. 2023

What we learned today

- Overview of tumor angiogenesis — both phenotypic and functional aspects
- Mechanisms of tumor vascularization, including vascular co-option
- Effects of angiogenesis inhibitors and mechanisms of therapeutic resistance
- Concept of vascular normalization and its impact on immune stimulation
- Role of vascular normalization via ANGPT2 targeting in reducing T-cell exclusion and enhancing immunotherapy efficacy (Kim Lab)

Vessel co-option and angiogenic switch

Ang2 or ANGPT2: angiopoietin-2
VEGF: vascular endothelial growth factor



Holash et al., Science 1999